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Guidelines for Surface Water Quality

Vol. 1 Inorganic Chemical Substances

COPPER

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Contents

	Page
ABSTRACT	v
RÉSUMÉ	v
ACKNOWLEDGMENTS	vi
RECOMMENDED WATER QUALITY OBJECTIVES FOR COPPER	vii
SOURCES, OCCURRENCE AND FORM	1
Natural distribution of copper	1
Production, consumption and uses of copper	2
Pathways for entering the environment	2
Forms of copper in the environment	5
RATIONALE	11
Raw public water supplies	11
Copper in the human body	11
Copper deficiency	11
Acute toxicity of copper	12
Chronic toxicity of copper	12
Interactions between copper and other metals in humans and laboratory animals	13
Recommended limit for drinking water	13
Aquatic life and wildlife	14
Accumulation of copper by algae and aquatic macrophytes	14
Intracellular distribution of copper	15
Toxicity of copper to algae	15
Effect of copper on photosynthesis, respiration and growth	16
Effects of pH on copper toxicity—algae	17
Effects of mixtures of contaminants—plants	18
Macrophytes	19
Accumulation of copper by macroinvertebrates	19
Toxicity of copper to invertebrates	20
Avoidance of copper—invertebrates	23
Hardness—invertebrates	23
Tolerance to heat after exposure to copper	23
Toxicity of mixtures of metals to invertebrates	23
Field study—invertebrates	23
Toxicity of copper to amphibians	24
Accumulation of copper in fish	24
Toxicity of copper to fish	25
Pre-exposure to copper	25
Chronic toxicity and sublethal effects—fish	25
Reproduction—fish	25
Osmoregulation—fish	27
Respiration—fish	28
Feeding behaviour—fish	28
Hematology—fish	28
Endocrinology—fish	29
Growth—fish	29

Contents (cont.)

Page

Some factors affecting the toxicity of copper in fish 30
The effect of fish size on toxicity 30
pH 30
Temperature 30
Hardness 30
Toxicity of copper in the presence of other pollutants 31
Avoidance of copper 33
Immune response to infection; effect of parasites 34
Field studies—fish 34
Wildlife 35
Recommended limit for aquatic life and wildlife 36
Water supply for livestock 36
Copper in the animal body 36
Copper deficiency 37
Acute toxicity of copper 38
Chronic toxicity of copper 38
The interaction of copper with other elements and compounds in livestock 38
Recommended limit for copper in livestock drinking water 39
Water supply for irrigation 40
Copper in soils and plants 40
Copper deficiency 42
Acute toxicity to plants 43
Chronic toxicity to plants 43
Recommended limit for irrigation water 44
Recreation and aesthetics 45
Recommended limit for recreational waters 45
Industrial water supplies 45
Recommended limit for industry 45

REFERENCES 45

Tables

1. Global summary of copper sources to the environment 2
2. Approximate copper emissions to the atmosphere 3
3. The short-term lethal tolerance of freshwater invertebrates 21
4. Sublethal toxicity of copper to fish 25
5. Acute toxicity of copper to fish 31

Abstract

A literature survey was carried out on the toxic effects of copper and copper compounds on human health, aquatic life, plants and livestock. The information is summarized in this publication. From it, maximum copper concentrations in water at which toxic effects will not appear are recommended.

Résumé

On a effectué une étude de la documentation portant sur les effets toxiques du cuivre et de composés du cuivre sur les humains, les organismes aquatiques, les plantes et le bétail. Ces données sont résumées dans cette publication. En se basant sur ces données, on propose des valeurs des concentrations maximales de cuivre dans l'eau auxquelles on n'observe pas d'effets toxiques.

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RECOMMENDED WATER QUALITY OBJECTIVES FOR COPPER

Uses	Recommended objective (as total copper)
Raw public water supply	0.5 mg/L
Aquatic life and wildlife	0.002 mg/L
Agricultural water supply	
Livestock watering	1 mg/L (sheep and cattle present) 5 mg/L (all other cases)
Irrigation	0.2 mg/L (continuous use on all soils or intermittent use for copper-sensitive plants, e.g. vegetables) 1 mg/L (less sensitive plants, e.g. cereals) 5 mg/L (short-term use)
Recreation and aesthetics	
Recreational waters	0.5 mg/L
Industrial water supplies	
Food processing	0.5 mg/L

Guidelines for Surface Water Quality
Vol. 1. Inorganic Chemical Substances. Copper

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SOURCES, OCCURRENCE AND FORM

Natural Distribution of Copper

The crustal (igneous rocks) average concentration of copper has been estimated at 55 mg/kg (other estimates range from 23.6 to 47 mg/kg – Cox 1979). This value is higher than that for other well-known elements such as cobalt or lead; it is lower, however, than that for zinc, nickel or chromium. Copper ranks 25th in abundance among the elements present in the earth's crust (Taylor 1964). The average copper concentration in sedimentary rocks is lower: 45 mg/kg in shales, 5 mg/kg in sandstones and 4 mg/kg in limestones (Turekian and Wedepohl 1961; Bowen 1966, p. 16ff.). Copper has a tendency to concentrate in sedimentary manganese oxide and in clay mineral fractions, especially those rich in organic carbon. More than 99.9% of the copper carried to the oceans is precipitated, mostly with the clays and partially with manganese oxides (NAS 1977, p. 3). Deep-sea clay sediments contain an average of 250 mg/kg of copper (Turekian and Wedepohl 1961).

Although copper is sometimes found in a very pure state as native copper (around Lake Superior, for example), 80% of the copper production is from low-grade minerals containing 2% or less of the metal. Of the two main classes of copper ore, sulphides are more important commercially than the oxides. Copper is often found in association with iron, nickel, zinc, arsenic, antimony, bismuth, molybdenum, silver, gold and the platinum metals (Massey 1973).

More than 160 copper-containing minerals have been described. A few examples are chalcopyrite – $CuFeS_2$, chalcocite – Cu_2S, covellite – CuS, cuprite – Cu_2O, tenorite – CuO, and malachite – $Cu_2CO_3(OH)_2$ (Massey 1973).

The concentration of copper in soils ranges between 2 and 100 mg/kg (dry weight) with a mean value of 20 mg/kg (Bowen 1966, p. 39); Thornton (1979) quoted concentrations of up to 175 mg/kg in soils from India and up to 980 mg/kg in soils from USSR. The range of copper concentration in soil solutions, i.e. the water held in soil capillaries and which may constitute up to 50% of natural soils by weight, was 0.01 to 0.06 mg/L (Bowen 1966, p. 31). Shacklette *et al.* (1971) found a range of <1 to 300 mg/kg and an average of 18 mg Cu/kg in soils in the United States. In the Sudbury (Ontario) area, where one of the world's major deposits of nickel and copper is located, the concentration of copper in soil reached levels over 2000 mg/kg in the top 5 cm of soil within 1 km from the smelter. As with other metals, the copper concentration seemed to be falling off with the square or the logarithm of the distance from the smelter, a characteristic pattern for airborne particles (Costescu and Hutchinson 1972; Hutchinson and Whitby 1974; Hutchinson 1979). The increased acidity of these soils (due to wet and dry precipitation contaminated by SO_2) increased metal mobility and availability to plants (Hutchinson and Whitby 1977).

The concentration of copper in seawater has been variously estimated at 0.0005 mg/L to 0.003 mg/L (Brewer and Spencer 1975; Bowen 1966, pp. 19–20). The concentration of copper in river waters has been estimated at 0.0006 to 0.4 mg/L with a median value of 0.01 mg/L (Bowen 1966, p. 31). Copper concentrations in water samples collected in the Amazon River basin ranged from approximately 0.0004 to 0.0024 mg/L (Boyle 1979). The results for "extractable" copper collected from Canadian rivers between 1972 and 1977 and stored in the National Water Quality Data Bank (NAQUADAT) ranged from 0.001 mg/L to approximately 0.5 mg/L, with 90% of the over 12 000 results being below the value of 0.011 mg/L. A few isolated results were greater than 0.5 mg/L. Similarly in lakes, the extractable copper concentration ranged between 0.001 mg/L and approximately 0.1 mg/L. The 90th percentile of over 1400 results was 0.009 mg/L (Environment Canada 1977).

The copper concentration in sediments from the Great Lakes ranged from 0.5 to 277.5 mg/kg, with patterns of distribution and levels closely related to the urban-industrial activity in the region (Hutchinson and Fitchko 1974). Hall *et al.* (1976) reported the same pattern of copper levels in sediments in a study of the Brunette drainage system in British Columbia. They found that the copper sediment concentrations in an industrial area had a median value of 83 mg/kg (dry weight), while in a residential and green-space area the median value was only 14.6 mg/kg. The overall range of copper sediment concentrations was 11 to 1765 mg/kg (dry weight). In

the Ottawa River, the acid extractable copper concentrations in the sediment ranged between non-detectable and 179 mg/kg (air dry) with a median value of 22.5 mg/kg. In the Rideau River the range was non-detectable to 121 mg/kg with a median concentration of 14 mg/kg (Oliver and Agemian 1974). In a survey in an area of the United Kingdom free from industrial and sewage pollution (Solway estuary) the copper concentration in sediments ranged between 1.9 and 16.8 mg/kg dry weight (Taylor 1976).

Production Consumption and Uses of Copper

Copper ores are mined by both open pit and underground mining. Economics require a grade ore containing at least 0.7% to 6% copper for underground mining, whereas open pit mining can operate with ores yielding as little as 0.4% of copper (Massey 1973).

The 1975 world's mine production of copper was 7 447 040 tonnes, a drop of 5.2% from the 1974 production. The main producers were the United States with 17.2% of the world's production, USSR (16.1%), Chile (11.1%) and Canada (9.7%), followed by Zambia, Zaire, etc. (Wood 1976).

The 1975 world's production of refined copper was 8 207 440 tonnes, a drop of 7.4% from the 1974 production. The main producers of refined copper were the United States (19.6% of the world's production), USSR (16.4%), Japan (10.0%), followed by Zambia, Chile, Canada, etc. (Wood 1976).

The 1975 world's consumption was 7 480 970 tonnes, representing a decrease of 11.0% from the 1974 consumption. The main consumers were the United States with 18.7% of the total, USSR (16.0%) and Japan (10.8%) (Wood 1976).

The Canadian copper mine production in 1975 was 724 050 tonnes, a decrease of 12.2% from the 1974 level. The production was concentrated in four provinces: Ontario (36.6%), British Columbia (33.3%), Quebec (16.3%) and Manitoba (8.9%) (Wood 1976).

The 1975 production of refined copper in Canada was 583 300 tonnes, a decrease of 5.4% from the 1974 level (Wood 1976). Canada exports a good part of its production as copper ores and concentrates. In 1975, 314 570 tonnes of copper were exported as ores and concentrates, mainly to Japan (72%). In the same year Canada exported 319 570 tonnes of refined copper to various countries: the United States, United Kingdom, West Germany, France, etc. The copper smelters are located in Ontario, Quebec and Manitoba. The copper refineries are located in Montreal (Quebec) and Copper Cliff (Ontario) (Wood 1976).

Copper metal and its compounds have been used by man since prehistoric times. It was the first metal harnessed by man and estimates have it as being second only to iron in its usefulness to the human race. Today the main uses of copper are in the production of electrical wire, piping, and the manufacture of a series of alloys (e.g., bronze, brass) used in different applications (Massey 1973; Wood 1976).

Pathways for Entering the Environment

Table 1 gives a global summary of copper sources to the environment. Bertine and Goldberg (1971) have estimated that approximately 80 000 to 250 000 tonnes of copper enter the environment annually as a result of weathering mobilization.

Table 1. Global Summary of Copper Sources to the Environment

	Amount released to the environment '000 tonnes/year
Natural weathering	80–250
Processing of copper ore	37
Manufacturing of iron and steel	10.5
Coal burning	0.3–2.1
Sewage	42
Lost by use	31
Total	≈200–373

There are several possible sources of copper emission to the atmosphere. Nriagu (1979a, b) estimated that the total copper emissions to the atmosphere amounted to 74 500 tonnes/year of which approximately 25% were from natural sources (windblown dusts, vegetation exudates, volcanic emissions, sea salt sprays) and the rest from anthropogenic sources (metal production, wood and fossil fuel combustion, waste incineration). An enrichment factor of 93 for the copper concentration in air over the crustal abundance was found over the South Pole and it was concluded that this might be due to selective volatilization through such natural processes as volcanism (Zoller *et al.* 1974; Weiss *et al.* 1975). Using a different method of calculation, Duce *et al.* (1975) found an enrichment factor of 120 for copper over the North Atlantic.

Table 2. Approximate Copper Emissions to the Atmosphere (1975 production and consumption data)

Sector	Emission factor	% Control[1]	World production[2] '000 tonnes	World emission	Can. production[2] '000 tonnes	Can. emission
Manufacturing Ind.	5 kg Cu/tonne Cu[1]		7 447	37	724	3.6
Pig iron	0.375 kg Cu/tonne iron[1]	97	468 000	5.3	9 310	0.1
Steel, open hearth	0.0255 kg Cu/tonne steel[1] (controlled)	-	163 000[a]	4.2	3 080	0.08
Steel, basic oxygen	0.0115 kg Cu/tonne steel[1]	90	326 000[b]	0.4	7 305	0.008
Steel, electric furnace	0.016 kg Cu/tonne steel[1]	77	163 000[a]	0.6	2 640	0.01
Coal burning	0.002 to 0.015 kg Cu/tonne coal[3,4]	90	1 427 000[c]	0.3 to 2.1	10 700	<0.02
Wood burning	0.018 kg/tonne wood[5]	-	640 000[5]	11.5	7 500	0.1
				59.3		3.9

[a]Estimated at 25% of world raw steel production.
[b]Estimated at 50% of world raw steel production.
[c]Estimated at 50% of 1974 world production. The other half assumed to be used in the manufacture of coke (Bertine and Goldberg 1971).

References:
[1] Davis, W.E. 1972.
[2] Metal Statistics 1976, p. 203.
[3] Bertine, K.K. and Edward D. Goldberg 1971.
[4] Theis, Thomas L. 1975.
[5] Nriagu, Jerome O. 1979b.

They also concluded that this high enrichment factor might be due to high-temperature processes of natural (e.g., volcanism) or anthropogenic origin. In another study (Chester and Stoner 1974) the copper concentrations in the particulate matter in the lower troposphere collected over a series of the world's oceans and coastal localities varied between 23 and 490 mg/kg with an average of 168 mg/kg. This represented enrichment factors over the crustal abundance of 0.4 to 11 with an average of 3.4. In "open-ocean" regions enrichment factors of 6 were found, leading to the conclusion that anthropogenic copper inputs must exist in addition to natural sources. A table of atmospheric copper concentrations at remote locations compiled by Nriagu (1979b) shows a concentration range of 0.029 to 12 ng Cu/m^3 (or approximately 0.024 to 10 μg/kg) and enrichment factors of 0.6 to 546. The calculated pre-industrial atmospheric concentration of copper, based on the estimated natural emission rate and some other assumptions, is 0.06 ng/m^3. The same reference quotes a range of 5 to 50 ng/m^3 for the average atmospheric copper concentration in rural areas and 30 to 200 ng/m^3 in urban areas.

According to Davis (1972) and Nriagu (1979b), the largest source of copper emissions to the atmosphere is the metallurgical processing during primary copper production (see Table 1). Using 1969 data, this source accounted for 57.1% of the estimated atmospheric copper emissions in the United States. Other important sources were iron and steel production (28.3%), and coal burning (6.8%).

Using the same emission factors as Davis (1972) and some world and Canadian production figures for the year 1975, estimates of the main copper emissions to the atmosphere have been summarized in Table 2. Oil combustion does not seem to be a major source of copper to the atmosphere. The copper concentration in oil was estimated at 0.14 mg/kg (Bertine and Goldberg 1971). The world oil consumption in 1974 was approximately 2.7 billion tonnes (Skinner 1976), which means that approximately 380 tonnes of copper were released to the atmosphere on a global basis.

From air, copper can reach the aquatic environment by wet or dry deposition. Nriagu (1979a) estimated that of the 74 500 tonnes which are emitted to the atmosphere annually, from both natural and anthropogenic sources, approximately 83% (61 800 tonnes) are deposited on land and the rest in the oceans. In a study of the Walker Branch Watershed (Tennessee) (Lindberg *et al.* 1975) it was estimated that in the case of copper the dry deposition represented 24% of the total deposition. In the same study it was also found that the watershed retained copper very effectively (82% to 84%) together with other metals (e.g., Pb, Cd). The measured copper input in rainfall ranged

from 0.56 to 5.2 mg/m^2·month. Shiomi and Kuntz (1973) estimated the mean monthly areal deposition rates in the Lake Ontario basin at 0.33 mg/m^2 or 4 mg/m^2·a, giving an annual lake loading of 78 tonnes of copper. Kuntz (1978) estimated the atmospheric copper loading to Lake Erie at 120 tonnes/year. Also in the Great Lakes region, it was estimated that the atmospheric loading of copper to Lake Superior was 370 tonnes/year and to Lake Huron, 760 tonnes/year (Acres Consulting Services Ltd. and Applied Earth Science Consultants Inc. 1975; Swanson 1976).

Other estimates of copper deposition (rainwater and dry fallout) rates ranged from 19 mg/m^2·a at a rural site to 3070 mg/m^2·a at sites near a copper smelter in Wollongong, Australia (Beavington 1977). A compilation (Nriagu 1979b) of the total copper deposition rate at various locations in Ontario during 1970–75 shows a range of 2.7 mg/m^2·a (Traverse Lake) to 348 mg/m^2·a (Sudbury North). The total copper concentration in precipitation samples ranged from 4.1 to 515 μg/L. Another study estimated that near Sudbury (Ontario) the copper deposition ranged from about 65 mg/m^2·a to 1700 mg/m^2·a at approximately 1 km from the smelter (Hutchinson and Whitby 1977). The average deposition rate around St. Andrews, New Brunswick, was calculated to be approximately 6.7 mg/m^2·a (Zitko and Carson 1971).

The copper concentration in precipitation collected at a station at Lumsden Lake near Sudbury, Ontario, ranged between 0.009 and 0.013 mg/L, while the surface snow contained 0.024 to 0.031 mg/L of copper (Beamish and Van Loon 1977). The smelters close to Lumsden Lake emit large quantities of copper and other substances (nickel, SO_2). Snow collected near Thompson, Manitoba, near a nickel smelting operation contained between 0.09 mg Cu/kg at a distance of 8.5 km from the smokestack and 6.08 mg Cu/kg at 1.6 km from the smokestack (Hocking and Blauel 1977). In the vicinity of St. Andrews, New Brunswick, the rain and snow contained 0.003 to 0.013 mg Cu/L (Zitko and Carson 1971). The mean copper concentration in precipitation over the Lake Ontario basin was 0.006 mg/L and over Lake Erie, 0.002 to 0.004 mg/L (geometric mean as copper extractable) (Shiomi and Kuntz 1973; Kuntz 1978).

According to Nriagu (1979b), on a global basis the atmospheric deposition represents only 0.5% of the estimated 6.33 million tonnes of copper entering the oceans each year. Another 0.5% came from waste disposal, while the major part (99%) came from land runoff. Local situations can be very different from this global picture. For example, Robbins *et al.* (1972) concluded that the mean daily tributary input of copper to Lake Michigan was 0.38 tonnes, while daily emissions into the atmosphere from sources in Chicago, Milwaukee, and northwest Indiana were 9 tonnes.

Copper can also reach the aquatic environment from a variety of other sources: metal process pickling baths, copper and brass plating baths, wastewater from the alkaline Bemberg rayon process, pulp and paper mills, petroleum refining, metal works and foundries, mine water, mine tailings ponds, and acid mine drainage (from lead mining, for example). Sittig (1975) has summarized the copper concentration found in the wastewaters from some of these sources. They can reach averages of up to 900 mg Cu/L in the wastewater from processes such as brass rod and wire, and copper wire mills.

Allan (1975) discussed some of the high copper concentrations (21 000 mg/kg) found in lake sediments in mining and ore processing areas of Canada. Streams draining metal-mining areas generally contain higher concentrations of various metals than streams draining coal-mining areas. Most of the metal deposits consist of sulphide ores. Their oxidation, especially that of pyrite, yields waters of low pH which in turn dissolve other metal sulphides and release a series of metals to surface waters. The copper concentration in metal-mining areas of Colorado, for example, averaged 0.180 mg/L (with a standard deviation s = 0.920 mg/L), while the average copper concentration was only 0.0017 mg/L (s = 0.0012 mg/L) in control areas and 0.0023 mg/L (s = 0.0020 mg/L) in coal-mining areas (Wentz 1974). The concentrations of Zn, Cd, Co, Mn, and Ni were also greater than those in the control area.

An interesting result was obtained by Dreesen *et al.* (1977). They found that less than 7.9% of the copper was extracted from the electrostatic precipitator ash of a coal-fired power plant by a series of 1.0 mol/L or more dilute acid solutions (HCl, HNO_3, citric acid), or water. This very low solubility explained why the effluent waters from the power plant showed no increase in the copper concentration when compared with the influent waters.

Humic acid, found in soils or water, could be very active in mobilizing metals from minerals. Thus, for example, a solution of 0.1% w/v humic acid acting on mineral grains ranging in size from 297 to 590 μm dissolved 0.140 mg of copper from chalcopyrite in 24 h, whereas distilled water dissolved only 0.010 mg in the same period. The same 0.1% humic acid solution mobilized approximately 15 mg of copper in 24 h from chalcocite and distilled water dissolved only 0.2 mg (Baker 1973). Dissolved copper concentrations in Torch Lake (Michigan), which served as a deposit site for copper mine tailings for over 100 years, ranged between 0.073 and 0.100 mg/L.

The corresponding total copper concentrations ranged between 0.089 to 0.117 mg/L. The bottom sediments had copper concentrations ranging between 1310 and 3860 mg/kg (dry weight) (Lopez and Lee 1977).

Clarke (1974, p. 24) in his review of the literature on the effects of effluents from metal mines in Canada gives examples of copper concentrations in mine effluents (exploration and ore processing). These concentrations ranged up to 8.3 mg/L with no treatment and up to 1.1 mg/L after treatment.

Analysis of leaf litter and soil samples in the "New Lead Belt" of Southeast Missouri showed that higher than background concentrations of copper could be found for a distance of up to approximately 16 km from the smelter. The district, heavily forested with oak, had background copper concentrations of 1.5 to 4 mg/kg in soil and 6 to 11 mg/kg in leaf litter (dry weight). Near the smelter the copper concentration in the leaf litter reached 2200 mg/kg and 100 mg/kg (dry weight) in the first 5 mm of the soil (Bolter *et al.* 1975).

Other man-made pathways by which copper enters the environment are sewage effluents and used copper, which is not recovered as scrap. Galloway (1972) estimated that 42 000 tonnes/year of copper are released into the environment through sewage. It is very difficult to estimate the amount of used copper which is lost to the environment. As an order of magnitude one can probably assume that 0.5% of the consumption is lost 10 years later. Thus, for example, the copper consumption in 1967 was 6.2 million tonnes, which leads to a calculated loss figure in 1977 of approximately 31 000 tonnes (Metal Statistics 1976, p. 29). The pathways by which copper enters the environment and the respective amounts have been summarized in Table 2. Agricultural uses (fertilizers, fodder, and pesticides) seem to be another major pathway. In the Netherlands, Hueck (1975) estimated that this source released about twice as much as the plating and electronics industry together.

For the Rhine River, Hueck (1975) estimated that 90% of the copper concentration originated from anthropogenic sources and only 10% from "natural" sources. For the northern half of Chesapeake Bay, Maryland, Helz (1976) estimated that the major source of copper (~37%) was direct industrial discharge and that approximately 67% of the copper input to the bay was of anthropogenic origin.

Netzer *et al.* (1975) sampled effluents from textile mill dyehouses and found copper in half of the samples ranging from 0.12 to 4.20 mg/L. Data collected in New York City (Klein *et al.* 1974) showed high average copper concentrations in wastewaters from such industries as soft drinks and flavouring syrup (2.04 mg/L), ice cream (2.7 mg/L), laundry (1.70 mg/L), fur dressing and dyeing (7.04 mg/L). The residential wastewater had average copper concentrations ranging from 0.11 to 0.33 mg/L (the U.S. drinking water standard is 1.0 mg/L). The average copper concentration in the runoff from the city streets during rains was 0.46 mg/L in New York City and 0.176 mg/L in Lodi, New Jersey (Klein *et al.* 1974; Wilber and Hunter 1977). Considering the respective volumes of water, Klein *et al.* (1974) estimated that 47% of the total copper load entering New York City wastewater treatment plants is of residential origin, about 14% from runoff and 12% from electroplaters.

For a series of locations in Lakes Huron, Erie and Ontario, Kemp and Thomas (1976) estimated that the anthropogenic input of copper ranged between 33% and 69% of the total copper input at a given location. In absolute values the estimated total, i.e., natural plus anthropogenic, copper input ranged between 6 and 151 $mg/m^2 \cdot a$. The anthropogenic loadings paralleled the population of the area and also reflected the pressure of heavy industry at certain locations.

Forms of Copper in the Environment

Copper is found in nature as Cu(0) (metal), Cu(I) and Cu(II). A Cu(III) ion is also known. More than 160 minerals containing copper have been described. Chalcopyrite ($CuFeS_2$) is thought to account for 50% of world's copper deposits. Other copper-bearing ores are chalcocite (Cu_2S), cuprite (Cu_2O), malachite ($Cu_2CO_3(OH)_2$), etc. (Massey 1973, p. 4ff).

From thermodynamics it can be shown that in aqueous solutions and crystals, Cu(II) is the more stable ion contrasting with the gaseous phase in which Cu(I) is the preferred oxidation state. Similarly, in non-aqueous, non-complexing solvents (e.g., propan-1-ol) the cuprous state, Cu(I), is the more stable form. In the presence of ligands both Cu(I) and Cu(II) states can exist, depending on the affinity of the ligand for one or the other ion. For example, 1,2-diaminoethane stabilizes Cu(II) ion, while longer chain diamines, where chelation to Cu(II) is not possible, stabilize the cuprous state (Massey 1973, p. 17).

In the aquatic environment copper may be present in (i) solution, as cupric ion or complexed (carbonates, cyanides, amino acids, polypeptides, humic substances), (ii) suspended sediments, as precipitates (hydroxide complexes, phosphates, sulphides) or sorbed on other particles,

and (iii) bottom sediments, again as precipitates or sorbed on other bottom materials (Clarke 1974; Stiff 1971). The percentage of copper present in each of these forms depends on the pH and other inorganic and organic substances present in the water. Attempts to calculate the distribution of copper in aquatic systems have yielded different results, mainly because of the uncertainties in the thermodynamic data (e.g. hydrolysis constants, complex formation equilibria constants, solubility) and in the adsorption characteristics of Cu(II) complexes (Leckie and Davis 1979).

Elzerman (1977) found that copper, together with other metals (Cd, Zn, Pb) and some organic compounds, has a tendency to concentrate in the surface microlayer (the upper 0.3 mm of the water surface). For copper the surface enrichment factor over the bulk concentration was 2.6. Organic compounds, particulate matter, and atmospheric particulate matter played significant roles in this surface enrichment process.

Andrew (1976) showed that the toxicity of copper to fish and *Daphnia* correlated well with cupric ion activity measured using an ion specific electrode. Neither the dissolved copper (defined as that passing through a 0.45-μm filter) nor total copper, both measured by atomic absorption, explained the results of the toxicity experiments. In one experiment, for example, at a dissolved copper concentration of 0.215 mg/L, survival time of *Daphnia* was approximately 50 min, and at a dissolved copper concentration of 0.278 mg/L the survival time was 544 min. The corresponding Cu^{2+} activities were 0.026 mg/L and 0.004 mg/L, respectively. Most of the dissolved copper was in the form of dissolved but undissociated $CuCO_3$. The survival time of rainbow trout in hard water was also related to the cupric ion activity measured by specific ion electrode (Shaw and Brown 1974). The same conclusion was also reached by other workers in this field (Pagenkopf *et al.* 1974; Sunda and Guillard 1976). For example, Sunda and Guillard (1976) showed that growth rate inhibition and copper content of cells in culture experiments with *Thalassiosira pseudonana* in highly chelated seawater media were related to the cupric ion activity and not to the total copper concentration. The cupric ion activity could be changed independently of total copper concentration by varying the chelating agent concentration and the pH.

In studies with algae, in some lakes located in southwest Manitoba, Wagemann and Barica (1979) found that the effectiveness of copper as an algicide under the prevailing conditions of high pH, ranging from 8.0 to 9.4, and high concentrations of dissolved organic matter could be explained better if, in addition to Cu^{2+}, the species $CuOH^{+}$ and $Cu(OH)_3$ were also considered to be toxic. They called the sum of the three species [Cu^{2+} + $CuOH^{+}$ + $Cu(OH)_2$] "total toxic copper."

The toxicity of copper and zinc to juvenile Atlantic salmon was reduced by the presence of humic acids (Cook and Cote 1972). Similar results were obtained in experiments conducted with guppies: the toxicity of copper was reduced in the presence of NTA, EDTA, humic acid, and glycine (Chynoweth *et al.* 1976). Lewis *et al.* (1972), in studying the Cu(II) toxicity to *Euchaeta japonica* (Copepoda, Calanoida) in seawater, found that toxicity decreased when water-soluble compounds of unknown composition but with soil-like characteristics (humic acids, soil extracts) were added to the water-copper solutions. Data quoted by Singer (1977) showed that the growth of cultures of *Oocystis marssonii* and *Chlorella vulgaris* was dependent on the concentration of free Cu^{2+} and not of soluble copper. In highly alkaline waters with a minimum of soluble complexing or chelating agents present, measurements of dissolved or "labile" (measured by anodic stripping voltammetry-ASV) copper may give a fair approximation to the biologically active form (Andrew 1976).

The direct toxicity of copper to fish should be determinable from the concentration of copper measured by the cupric ion specific electrode. Brown *et al.* (1974) concluded that measurements of both soluble and total copper in surface waters overestimate the values of the available concentrations to fish. In their estimate, 40% to 90% of the total copper might be associated with particulate material and, quoting Stiff (1971), 96% to 100% of the soluble copper might be present in the form of "nontoxic" complexes.

The type of ligand plays a major role in the biological availability of metals, for example, copper citrate is toxic to aquatic organisms, whereas copper-EDTA complex is not (Fitzgerald and Faust 1965). In general, copper forms more stable complexes with natural organic compounds than does Cd, Pb, or Zn (Hart and Davies 1977).

Chau and Lum-Shue-Chan (1974) have pointed out that in natural waters metal ions and miscellaneous organic compounds can form complexes as well as organo-metallic compounds of the metal-carbon bonding type, and that in addition, metals can be strongly adsorbed on colloidal particles. By differential pulsed anodic stripping voltammetry (DPASV) they measured the "labile" metal, which included the ionic part, the acetate-exchangeable metal, and part of the complexed metal. The other part, i.e. the strongly bound metal, was defined as the total metal minus the "labile" metal.

Copper (also cadmium, zinc, and lead) was measured by DPASV in water samples from the North Sea at pH 8.1 and from the Rhine River at pH 7.0. These concentrations were compared with the concentrations in the same samples at pH 2.7. The former represented 20% of the copper measured at the lower pH. The difference, at least in part, was probably due to the organically bound ions. At pH 2.7 the complexing capacity of the water was zero (Duinker and Kramer 1977).

Major shifts in copper equilibria and, therefore, in copper activity occur with changes in bicarbonate/carbonate, phosphate, and pH equilibria. Calculations carried out by Sylva (1976) showed that hydrolysis and precipitation reactions dominate the Cu(II) chemistry at pH values expected in most natural water systems and in the absence of significant levels of complexing organic compounds. The most significant process by which Cu(II) was removed from solution was the precipitation of malachite. The calculations also showed that the presence of organic complexing agents could alter the system, resulting in a highly variable specification of Cu(II) not only from system to system but also within one system over a period of time. Williams and Baldwin (1976) concluded that in the surface waters of the Southern California Bight the concentration of the cupric ions was controlled primarily by complexation with inorganic ligands and secondarily by organic chelating agents. Model calculations have shown that in fresh waters almost all copper (and mercury) is complexed by humic compounds (Mantoura *et al.* 1978).

Calculations and experiments carried out in lakes located in southwestern Manitoba showed that 78% to 98% of the dissolved copper (0.45 μm) is in the form of $Cu(OH)_2$ and complexed with dissolved organic matter. The free Cu^{2+} ions concentration ranged from <0.02% to 0.37% of the dissolved copper. The pH of the lakes ranged from 8.0 to 9.4. When copper was added to the lake waters (as $CuSO_4 \cdot 5H_2O$), its dissolved concentration decreased according to first-order kinetics. The half-times for the disappearance of dissolved copper from the water column ranged from 1 to 2 days in most of the lakes, and a steady state was reached in approximately 10 days after the addition of $CuSO_4 \cdot 5H_2O$. The copper concentration immediately after the addition of copper sulphate was around 0.25 mg/L (Wagemann and Barica 1979).

Experiments showed that the complex formation of copper (also lead, cadmium, and zinc) increased with increased concentrations of water-soluble fulvic acid and organic acids derived from decaying leaf litter. Complexing also increased with increasing pH. The increased concentration of KCl did not significantly affect the formation of metallo-organic complexes of copper (Bolter and Butz 1975). Jackson (1977b) found a good positive correlation between the organic carbon content and the copper (also Hg, Zn, Cd, and Fe) concentration in sediments from Clay Lake and Ball Lake (Ontario).

Equilibrium calculations showed that in Torch Lake (Michigan), which served as a copper mine tailings deposit, the predominant copper species was $Cu(OH)^+$, followed by Cu^{2+} and then by complex copper carbonates (Lopez and Lee 1977). Hart and Davies (1977) found that copper in water samples from a polluted creek in Melbourne, Australia, was mostly (~90%) in labile forms. In the interstitial water of the sediment samples the copper was almost evenly distributed between labile species (~40%) and more strongly bound complexes (~45%). Stiff (1971) has shown that for a series of rivers in England, the ratio of soluble copper/total copper ranged from 0.12 to 0.57. Soluble copper was defined as that which passed through a 0.45-μm membrane filter and included free cupric ion, soluble complexes, some colloidal matter, and possibly some precipitates, as well as copper-containing clays in a finely divided state that would pass through the filter. Total copper was the sum of soluble copper and copper in particulate form. Amino acids, polypeptides, and bicarbonate ions played a significant role in the complexing of copper, together with some other compounds of unknown nature which gave some very stable, inert cyanide-like copper complexes.

It must be noted, however, that at the moment there is no satisfactory method available for separating various copper species present in the aquatic environment. Marvin *et al.* (1970), for example, have tested five different types of filters by measuring the concentration of copper in salt water and in laboratory-prepared solutions with bi-distilled water. The results obtained were erratic; both contamination from the filter and sorption of the copper by the filter contributed to this. Andelman (1973) quoted data from a Missouri creek which showed differences between 0.45-μm and 0.1-μm filters.

Ramamoorthy and Kushner (1975) have shown that in river water samples from the Ottawa area (Rideau Canal and Brewery Creek) metal ions of the form M^{2+} (Pb^{2+}, Cu^{2+}, Cd^{2+}) at concentrations of 1 mg/L or less were completely bound to compounds with molecular weights of less than 1400. At higher concentrations some of the metal ions were detected as "free ions" in the dissolved state. In waters with a TIC (total inorganic carbon) of approximately 20 mg/L or above, some of the binding capacity was attributed to HCO_3^- and CO_3^{2-} ions. Humic substances, whose molecular weights can range from 300 to more than 200 000, and fulvic

acid were also suggested as possible binding molecules. In fact, in the same study in water samples from the Ottawa River most of the copper, 82.6%, was bound to compounds with molecular weights greater than 45 000.

Marine humic substances had copper concentrations ranging from 600 to 4000 mg/kg and they contained 5% to 79% of the copper bound in marine sediments (Nissenbaum and Swaine 1976).

Ultrafiltration of metal (Cu, Pb, Cd, Zn) - humic acid solutions indicated that at pH 6, the metal ions were present almost exclusively in the fraction of size >3.2 nm. At pH 2, however, 100% of the metal ions were in the fraction of size <1.9 nm (Guy and Chakrabarti 1976). It was also determined that at pH >6, 50% of the copper was present in the particulate fraction and 50% of the copper was complexed. At pH 2, 100% of the copper was present as free ion (Guy *et al.* 1975). Copper in natural waters was found exclusively in two fractions, one >5.1 nm and the other <1.9 nm, in various proportions depending on the origin of the water sample (Guy and Chakrabarti 1976).

Samples of Hudson River water showed that over 60% of the copper was associated with particulate matter of >2 μm (Catanzaro 1976). In a study of the Yukon and Amazon Rivers, 3.3% and 6.9%, respectively, of the total copper was in the dissolved (0.2 μm) phase, the rest being found as suspended materials (oxide coating, in the crystal lattice or bound with particulate organic materials — Gibbs 1973). According to Gibbs (1977), on a global basis only 1%, or 61 300 tonnes, of the copper transported by rivers to the oceans is in soluble form. Of the rest, 85% was associated with the crystalline material, 4.5% with organic material, 5.7% was bound to the metal hydroxide coatings and 3.5% was adsorbed onto the suspended particulate.

The treatment of a Californian aqueduct with copper sulphate (for the control of phytoplankton and the alga *Cladophora*) at a rate of approximately 823 kg/km over a 4-year period did not lead to an increase in the copper concentration in water. The mean concentration of copper in the <20 μm sediment fraction in the untreated reach was 0.0298 mg/kg compared with 0.0597 mg/kg in the treated reach. Similarly, biotic samples (plants, clams) showed increased copper concentrations in the treated reach compared with the untreated reach (Fuller and Averett 1975). In a similar situation (algal control of Lake Perris reservoir in Southern California), Elder and Horne (1975) found that the added copper is converted eventually to particulate forms but that the conversion is relatively slow: after 4 weeks most of the copper was still in solution.

Payne and Pickering (1975), studying the interaction between Cu(II) solutions and kaolinite clay suspensions, found that at pH <5 protons successfully competed for adsorption sites and at pH <3.5 no Cu ion uptake occurred. The addition of various ligands changed this situation completely. At pH <5 the presence of the ligands led to an increased copper adsorption. At higher pH's some ligands (tartrate, gluconate) limited the amount of copper sorbed by the clay suspension. Similarly, when the ligand was in contact with the clay prior to the addition of copper ions, the amount of copper taken up was reduced. On the other hand, no adsorption occurred when clay was added to a solution containing preformed complex.

In a laboratory study with Cu^{2+} solutions to which MnO_2, bentonite, or solid humic acids were added it was found that the copper distribution depended on the pH of the suspension. At pH >6.0 half of the copper was sorbed onto the particulates and the other half was complexed in solution. At pH 3.8 to 6.0 some of the soluble copper was in free form. At pH 2.5 to 4.2 copper was desorbed from the particulates and finally at pH <2.5 all copper was in solution as free ion (Guy *et al.* 1975). Adsorption of copper, lead and zinc onto hydrous oxides and soils increased abruptly at pH 5 to 6. Anions enhanced metal adsorption at low pH values when adsorption was not likely to occur in their absence (Huang *et al.* 1977). The formation of sparingly soluble species $M_x(OH)_x^{x+}$ for divalent metal ions has been postulated by several authors (see, for example, Farrah and Pickering 1977). The presence of anions which form salts of low solubility should also have reduced the concentration of metal ions in solution.

Farrah and Pickering (1977) studied the removal of copper, lead, zinc and cadmium from aqueous solutions by clay suspensions. They concluded that the amount removed was a function of the nature of the clay (kaolinite, illite or montmorillonite), solution pH, concentration of competing cations, the nature and the concentration of any ligands present, and the order in which the various species, i.e., clay, competing ions and ligands came into contact. At initial concentrations of 1.7×10^{-4} mol/L (or approximately from 11 mg/L for Cu) the amount of metal adsorbed by clay increased linearly with pH in the range 3.5 to 6.5. In acidic solution (pH <5) most of the metal ions stayed in solution. The formation of stable cationic complexes could have prevented the precipitation of the sparingly soluble hydroxy species which become associated with the clay in alkaline media. Also, protonated ligands could have competed for adsorption sites, thus reducing the sorption of the metal ions on the clay materials. As the pH increased to about 7, most of the metal was precipitated and/or sorbed.

Lamb and Tollefson (1973) reported that the toxic effects of copper on the biological oxidation process occurring in a completely mixed activated sludge system were reduced by increasing the suspended solids concentration.

Frost and Griffin (1977) found that the removal of copper (also zinc and cadmium) from a municipal landfill leachate by kaolinite and montmorillonite increased with increased pH and concentration of the metal ions in the leachate. At low pH values adsorption was the dominant mechanism. As the value of the pH increased above 5.5 to 6.5, precipitation of species such as $Cu(OH)_2$, $Zn(OH)_2$, and $CdCO_3$ became the dominant mechanism for removal. Montmorillonite sorbed approximately five times more metal from solution than kaolinite.

The reverse process, i.e., the release of metals from the particulates, can take place by one of three mechanisms: (i) ion exchange, where a cation from the solution changes place with an adsorbed cation, (ii) solubilization of the matrix, and (iii) decomposition of the matrix. The release of metal from sediments via decomposition may be an important mechanism for the hydrous oxides of iron and manganese (Guy and Chakrabarti 1976).

In the study of the Yukon and Amazon Rivers quoted above (Gibbs 1973), 87.3% and 74.3% respectively of the total Cu was carried in the crystalline structure of the suspended materials; 3.8% and 8.1% as metallic coatings (i.e. precipitated and coprecipitated on solids), 3.3% and 5.8% in the solid biological materials, and 2.3% and 4.9% adsorbed on solids.

In an intensive study of the Susquehanna River (Troup and Bricker 1975) it was found that the total metal concentrations (Mn, Fe, Cd, Cr, Co, Cu, Ni, and Zn) were correlated with the discharge of the river and with the total suspended solids, thus showing that especially in periods of high discharge, most of the metals were carried in the suspended materials phase. Similarly, Jennett *et al.* (1977), when monitoring the water quality of several creeks in the Viburnum Trend or "New Lead Belt" of Missouri, a region of mining, milling and smelting operations, found that during peak runoff periods following a storm, the increases in the dissolved metal concentrations were very small, but the total concentrations increased abruptly and suspended solids increased significantly. Probably soil particles had been washed directly into the streams.

In the Mississippi River, Delfino (1977) found that the total copper concentration (also Fe, Mn, and Zn) was moderately correlated with discharge and total suspended solids (linear correlation coefficients, r's, of 0.57 and 0.50) and very strongly correlated with the concentration of Fe and Mn (r's of 0.94 and 0.92).

In another study it was found that 50% to 80% of the total copper present in river waters was in the suspended solids phase, possibly as oxide, sulphide, and malachite precipitates adsorbed on clay particles, or complexed by organic molecules. The removal of copper from the solution was fast. Starting with a solution containing 0.8 mg/L copper, 75% of the copper was removed from solution in two hours (Montgomery and Stiff 1971).

In tests with zinc and copper solutions prepared in the laboratory, it was shown that the toxicity to fish at a given total concentration of the metal ion decreased as the concentration of added suspended solids increased. The suspended solids which were added consisted of humus-tank solids from a sewage plant treating a detergent-free domestic sewage. If, however, the copper was measured as "soluble" (defined as that passing through a 0.45 μm average pore size membrane filter), then the toxicity to fish increased with increased concentrations of suspended solids. The concentration of the soluble copper (also zinc and cadmium) fell quickly after the preparation of the solids-metal salt solution mixture. The decay rate was pH dependent (Brown 1976).

Copper, together with other metals such as Mg, Pb, Zn, and Cd, was classified as an "enriched" element in sediments from Lake Erie (Kemp *et al.* 1976). The concentration in the sediment of these elements was higher than that expected from the soil composition in the basin and this was attributed to anthropogenic sources. Cline and Upchurch (1973) reported that the copper concentration in the upper 4.5 cm of a sediment sample increased exponentially over time by copper migration from the lower part. Walters *et al.* (1974) found that in sediments from Lake Erie, copper and other metals showed an enrichment tendency in the top 30 cm as compared with concentrations below this depth.

Copper and other metals have a tendency to concentrate in the fine particle size fraction of the bottom sediments. Thus, for example, in a study of the Susquehanna River Basin in New York State it was found that the total copper concentrations in silt and clay were 2.9 to 10.6 times greater, respectively, than the total copper concentration in sand (McDuffie *et al.* 1976). In the same study it was found that the sand transported only 1% of the "available" copper. Most of the copper, 53%, was transported in the dissolved phase; the silt and clay carried 15% and 31% of the total copper, respectively. Oliver (1973) suggested that the metal concentration in the

sediments was related to the surface area of the various particle sizes. Using results from the Ottawa and Rideau Rivers, he found statistically significant positive correlations between a series of metal concentrations (Cu, Pb, Ni, Co, Fe, Mn, Cr, but not Hg) and the surface area of the sediment fraction.

Grieve and Fletcher (1975, 1976) found strong positive correlations between Cu, Zn and Pb content and strong negative correlations between these three metals and the percentage of sand on the minus 80-mesh fraction of surficial sediments from the Fraser River Delta. The distribution of copper and other trace metals was closely related to the distribution of fine-grained material. Up to 80% of trace metal variance could be explained in terms of fluctuations in Fe, Mn and sand content of the sediment.

Most of the copper (79% to 86%), and also zinc and lead, in the sediments of the Saguenay Fjord was found in the detrital fraction (acid soluble). Strong, positive correlations were found between the metal (Cu, Zn, Pb, both detrital and non-detrital) and the mud (<53 μm) contents of the sediment (Loring 1976).

Laboratory and field results have also shown that at the sediment-water interface the highest copper concentrations (also manganese) were reached in water samples collected slightly below the interface (Plumb and Lee 1977). Copper concentrations in water samples collected down to approximately 9 cm below the water-sediment interface were 2 to 8 times greater than the copper concentrations in water samples collected at approximately 4 cm above the interface.

Copper was also concentrated in interstitial water of sediments collected from the western part of Lake Superior. The ratio of the copper concentration in the interstitial water of the top 2.5 cm of sediment to the mean value in lake water was 5.2 (Glass and Poldoski 1975).

A study of Indian Lake (Massachusetts) showed that 98% of the total copper inputs to the lake (consisting of ~90% of soluble copper from copper sulphate treatment) was retained in the system, most of it (~98%) in the sediments and the rest in the water column as soluble copper or as suspended sediments. The conversion of the soluble copper to particulate forms and their deposition on the lake bottom was approximately 90% completed by the 10th day. Only less than 1% of the total copper in the sediment was present in the interstitial water and only about 1% of the total copper could be removed by washing the sediments with deionized water (Symmes 1975).

Cline and Upchurch (1973) showed that copper in lake sediments has a very significant tendency (exponential increase through time) to migrate towards the upper 5 cm of the sediment, possibly by a bacterial mechanism.

Sanchez and Lee (1973) concluded that in Lake Monona (Madison, Wisconsin), a highly calcareous lake, the copper ions in solution react with calcium and magnesium carbonates in the sediments to form basic copper carbonate and to release calcium and magnesium ions into solution.

In anaerobic conditions copper is immobilized in the sediments and under aerobic conditions is released to the overlying water (Clarke 1974, p. 78ff.).

Hill and Helz (1973) suggested that copper can be extracted from sediments by chemical reaction with oxygen-rich, chlorinated cooling effluents from power plants. Under oxidizing conditions the concentration of copper (also Pb and Cd) in water in contact with sediments increased, probably due to the effect of the lower pH of the suspension (Khalid *et al.* 1978). This was confirmed by Lu and Chen (1977), who concluded that the metal migration between sediment and interfacial water was governed by the chemistry, mainly the redox potential (i.e., levels of dissolved oxygen and sulphur(-II)) of the immediate overlying water as well as that of interstitial water, and not by the sediment size. Under oxidizing conditions Cu (also Cd, Ni, Pb, and Zn) was released from the sediments.

Copper (and nickel) increased in concentration from 0.15 to 0.85 mg/L and the pH dropped from 6.5 to 4.0 in 28 days in water overlying lake sediments in "live" aerobic systems in laboratory aquaria. The copper was present as free cupric ions. In sterile anaerobic conditions the total copper concentration and the pH decreased slightly. In this case only less than 50% of the total copper was present as cupric ions. The results for the "live" anaerobic systems were intermediate between these two systems. The action of microorganisms and benthic organisms under aerobic or anaerobic conditions might lead to significant movement of metals from sediments into water (Stokes and Szokalo 1977).

Aeration of river water led to an increase in the concentration of dissolved copper (also zinc, lead, and chromium). This was interpreted as an indication that the organically bound heavy metals were being released (Müller and Schleichert 1977).

Experiments under static or flow-through conditions showed that a series of chelating agents (sodium tripoly-

phosphate-TPP, nitrilotriacetic acid-NTA, and ethylene diaminetetraacetic acid-EDTA) at concentrations of 0.2 to 5 mg/L did not change the copper concentration in water (tap water) above contaminated lake sediments as compared with controls (no chelating agents added). The addition of the chelating agents also had no influence on the copper concentration in chironomid adults, crayfish and rainbow trout (Barica *et al.* 1973).

RATIONALE

Raw Public Water Supplies

Copper in the Human Body

Copper is one of the essential elements in human metabolism. The main intake route for copper by humans is the diet. The copper concentration in selected food and beverages ranges from approximately 0.1 mg/L in whole milk to 44 mg/kg in calf liver. Other high copper content foods are oysters at 36 mg/kg; lobster, 22 mg/kg; beef liver, 21 mg/kg; hazelnuts, 13.5 mg/kg; and wheat germ, 13 mg/kg. Fresh fruit, most vegetables, eggs, and dairy products contain under 3 mg Cu/kg (NAS 1977, p. 29ff.). A normal diet can thus supply about 1 to 10 mg Cu/d or even more (NAS 1977, p. 29; Buhler 1973; Tremolieres *et al.* 1975). It has been estimated that the average daily copper intake from food in Canada is 2.2 mg/person; the average daily dietary requirement is approximately 1 to 3 mg/person (Méranger and Smith 1972; Neri 1976). Similarly, Neri (1976) estimated that the daily intake of copper from an average Canadian diet was 2 to 4 mg/person. In addition, the intake from drinking water was estimated at approximately 1.2 mg/person in soft water areas and 0.6 mg/person in hard water areas. In a survey of 28 countries, the average daily intake ranged from 1.6 mg/d in Taiwan to 3.4 mg/d in New Zealand. The copper concentration in the food surveyed ranged from 0.12 mg/kg in nuts to 7.13 mg/kg in beef and lamb (Schrauzer *et al.* 1977).

In a survey of metal concentrations in the drinking water in Seattle and Boston the mean copper concentration was 0.160 mg/L (Seattle) and 0.432 mg/L (Boston) in running-water samples. In standing-water samples the mean concentrations were higher: 0.450 mg/L (Seattle) and 0.494 mg/L (Boston). The maximum concentrations reached values of 1.670 mg/L in running-water samples and 2.410 mg/L in standing-water samples (Craun and McCabe 1975). Copper concentrations above 0.001 mg/L were found in 99% of the samples collected from 969 community water supply systems in nine different geographical areas of the United States (Craun and McCabe 1975). The maximum copper concentrations found in a 1975 survey of household drinking water in four locations of the eastern United States was 0.398 mg/L (Strain *et al.* 1975).

Approximately 40% to 70% (average 56%) of the orally ingested copper is retained; the rest is eliminated through the bile, feces, and urine (NAS 1977, p. 31; Tipton *et al.* 1966). It has been suggested that the percentage of copper absorbed decreases with increased copper intake. The absorption of copper takes place through the stomach, jejunum and the small intestine; then it is transported in plasma bound to albumin and perhaps to amino acids (C.H. Hill 1977; Buhler 1973). The whole-body content of copper has been estimated at 75 to 150 mg and the whole-body half-life at 80 days (Buhler 1973; Sandstead 1975). The whole-body, fat-free tissue copper concentration in adults has been quoted at 1.7 mg/kg and at 4.7 mg/kg in newborns (Underwood 1971).

The main copper depots are the muscle, liver and brain tissues, which contained (median values) 26.7, 11.3, and 8.1 mg of copper per whole organ or tissue, respectively (NAS 1977, p. 34). In units of concentration the organs with the highest copper values were liver, 9.2 to 46.8 mg/kg (dry weight); brain, 13.1 to 39.4 mg/kg; nails, 3.2 to 58.2 mg/kg; hair, 7.6 to 54.5 mg/kg; and stomach, 4.5 to 36.6 mg/kg (Smith 1967).

Several copper proteins have been isolated, such as ceruloplasmin, copper-chelatin, superoxide dismutase (cytocuprein), etc. (NAS 1977, p. 34). The biochemical function of copper is closely related to that of iron (Kirchgessner *et al.* 1977). The major physiological processes in which copper participates are the formation of blood and the utilization of iron in hemoglobin synthesis, the synthesis and cross-linking of elastin and collagen in the aorta and major blood vessels, and in oxidation processes by its presence in many oxidative enzymes (Perrin and Agarwal 1976; Horvath 1976; Masironi 1969; O'Dell 1976). Dilute copper solutions (e.g. 0.05% $CuSO_4$) have a low-grade antimicrobial activity, and metallic copper showed a gonococcicidal effect *in vitro* (NAS 1977, p. 52).

Pregnancy and estrogen treatment affect the normal copper metabolism (NAS 1977, p. 61).

Copper Deficiency

Although very rare, copper deficiency has been associated with anemia, neutropenia, diarrhea, demineralization of the bone and amyelination of the central nervous system of the newborn (NAS 1977, p. 38; Horvath 1976; Kirchgessner *et al.* 1977). Malnutrition and maleb-

sorption, especially in premature infants born with a smaller copper reserve in the liver, are the main causes of copper deficiency. The earliest detectable sign of copper depletion is a fall in serum copper and ceruloplasmin. The earliest clinical sign in patients with copper deficiency was neutropenia (Graham and Cordano 1976). These effects of copper deficiency can be reversed by addition of copper to the diet. Menkes' disease (trichopoliodystrophy), which also affects infants and which is characterized by kinky, depigmented hair; physical and mental retardation with degeneration of the brain; hypothermia; and eventually death, has also been linked to copper deficiency. In this case, however, neither oral or parenteral administration of copper reversed the symptoms.

In adults copper deficiency can occur when the intake or absorption of copper is drastically reduced or when, as in the case of prolonged administration of D-penicillamine, a negative copper balance occurs as a result of higher than usual copper elimination (NAS 1977, p. 39ff.).

Animal studies (guinea pigs, mice) indicated that copper deficiency causes congenital abnormalities, such as anemia, subcutaneous hemorrhages, skeletal anomalies, abnormal texture of hair, thin skin, etc., in the offspring. Some of these symptoms were corrected by a high-copper dietary intake (Hurley 1976). Copper deficiency also caused an increase in the embryonic deaths in rats and chicks (Ferm 1976). Copper deficiency in pregnant rats also increased the rate of fetal death *in utero* and the number of stillborns or pups consumed within a few days by their mother (Kirchgessner *et al.* 1977).

In a study carried out in South Wales, the copper concentration in the drinking water was significantly ($p = 0.05$) negatively correlated with neural tube defects (Roberts 1976).

Acute Toxicity of Copper

The ingestion of milligram quantities of copper induces vomiting and diarrhea. This may occur when acidic food or drink (vinegar, carbonated beverages, citrus juices) has been in prolonged contact with metal (e.g. soft drinks in contact overnight with the copper valves of drink-dispensing machines or drinks and foods sorted or prepared in bronze or brass vessels or mixers). The ingestion of gram quantities of ionic copper leads to ulcerations of the gastrointestinal mucosa, hemolysis, jaundice, hepatic necrosis and renal damage (NAS 1977, p. 40).

In a suicide case by ingestion of a 30% $CuSO_4$ solution, the copper concentrations in the brain, liver, stomach, and especially hair roots and urine increased significantly. According to Cross *et al.* (1976), hair levels reflect the blood levels at the time of growth.

Both acute and chronic effects on the histology of the testes and some interference with spermatogenesis were observed when rats were injected subcutaneously with a $CuSO_4$ solution in a single dose or daily doses for 10 to 30 days of 64 mg Cu/kg of body weight (Hoey 1966).

Chronic Toxicity of Copper

Chronic copper poisoning in man is very rare. This suggests that the human organism can adapt itself to a wide range of copper intake without detrimental effects. The copper concentration in the liver tissue of rats increased linearly with the dose when copper sulphate was administered intraperitoneally. Minimum doses of 0.3 and 0.5 mg Cu/d per kilogram of body weight for 18 and 12 weeks, respectively, were needed to elevate the hepatic levels. The renal, spleen, and lung levels also increased with the dose and the duration of loading. Brain and testes showed an early increase, but the values tended to plateau (Lal and Sourkes 1971). Young male rats fed on a diet containing 1 to 20 mg Cu/kg showed increased liver copper concentrations (Cerklewski and Forbes 1977).

Some of the chronic effects of copper toxicosis summarized by NAS (1977, p. 41) are pulmonary deposition and fibrosis, and granulomas and malignant liver tumours reported in some vineyard workers; acrodynia (pink disease) in an infant ingesting water containing 0.8 mg Cu/L; gingivitis, lichen planus, and eczematous dermatitis attributed to copper alloys used in dental and other prostheses.

Wilson's disease, a congenital condition consisting of hepatolenticular and hepatocerebral degeneration, has been associated with abnormal copper metabolism, i.e. a deficiency of the plasma copper – protein ceruloplasmin and an accumulation of copper in the liver, kidney, brain, and cornea (NAS 1977, p. 42; C.H. Hill 1977). Hepatic accumulation of copper has also been reported in children with biliary atresia and biliary cirrhosis (NAS 1977, p. 50).

Ferm (1976) quoted experimental results showing that copper in the citrate form had more of a teratogenic effect (malformation of the heart in mouse) than the copper in an uncomplexed state (sulphate).

The normal copper concentration of serum in adults ranges between 0.8 and 1.5 mg/L (NAS 1977, p. 35). Deviations from the normal concentrations have been used as diagnostic indicators in patients with acute leukemia and other lymphomas. Higher than normal serum copper

concentrations are also encountered in Hodgkin's disease, reticulum cell sarcoma, lymphosarcoma, and multiple myeloma (NAS 1977, p. 51).

Increased copper concentrations in serum or plasma were found in persons with atherosclerosis, hypertension, or myocardial infarction as compared to controls (Masironi 1976).

The connection between copper intake and cancer is inconclusive at the moment. Elevated serum copper levels have been observed in patients with lung, breast, cervical and other gynecological cancers, and certain carcinomas of the larynx and bladder. A direct correlation has been found between the dietary copper intake and mortalities from cancer of the intestine, breast, leukemia, and skin (Schrauzer *et al.* 1977). Experiments with laboratory animals have shown, however, that in certain cases copper also had anticarcinogenic properties.

Interactions between Copper and Other Metals in Humans and Laboratory Animals

A laboratory study with young male rats indicated that dietary copper at levels of 1 to 20 mg/kg increased lead toxicity as shown by elevated lead concentrations in the kidney and the increase in urinary excretion of δ-aminolevulinic acid (δ-ALA) (Cerklewski and Forbes 1977).

Klauder and Petering (1977) suggested that lead-induced anemia in rats, manifested by depression in hematocrit and hemoglobin levels, was due to an interference by lead of copper metabolism leading to copper deficiency. Supplementation of copper in the diet prevented this lead-induced anemia.

Results quoted by Evans and Cornatzer (1972) showed that zinc, cadmium, and ascorbic acid have an antagonistic effect on copper metabolism. For example, hepatic and plasma copper concentrations were greater in rats fed on a zinc-deficient diet as compared to control animals fed either on a stock or zinc-supplemented diet. In other words, high levels of zinc in the diet inhibited copper absorption, whereas dietary zinc deficiency resulted in increased copper absorption. With rats, zinc depressed copper uptake from the stomach and the duodenum but did not change the tissue distribution of copper. Competition between these metals for the binding sites of the protein metallothionein is probably the mechanism of their antagonism. Both copper and zinc contribute to the control of the blood lipid level (Masironi 1976) and it has been suggested that the dietary ratio of zinc to copper influences the metabolism of cholesterol in man (Sandstead 1975).

Cadmium depressed the copper uptake and the proportion of copper retained by the liver while increasing the proportions retained by blood, heart and spleen. Silver had no significant effect on copper uptake but it decreased the proportion of copper in the blood while increasing that in the liver. Similarly, mercury had no significant effect on the copper uptake but increased the proportion of copper retained by the kidneys (Van Campen 1966). Moffitt and Murphy (1973) suggested that either a deficient or a high copper intake diet might modify the metabolism and the toxicity of other toxic chemicals.

Nickel at a dietary level of 20 mg/kg compensated for the weight loss of rats due to copper deficiency. Nickel, however, did not prevent the decline in the activity of ceruloplasmin that occurs during periods of low copper intake (Spears and Hatfield 1977).

Recommended Limit for Drinking Water

The main route for copper intake by humans is via food consumption, with an average of approximately 2 mg/d, and drinking water (Neri 1976). The respiratory intake has been estimated at approximately 0.006 mg/d (Schroeder 1974). The estimated dietary requirements for copper range from 0.03 mg/d per kilogram of body weight in adults to 0.08 mg/d per kilogram of body weight in infants and children (Sandstead 1975).

Treatment of water by microstrainers, clarifiers, and filters removed an average of approximately 49% of the copper at an influent average concentration of 0.03 mg/L (maximum concentration, 0.160 mg/L) (Zemansky 1974). Results quoted by Netzer and Beszedits (1979) showed that conventional activated sludge treatment of a plant in Burlington, Ontario, reduced the total copper concentration from an average of 0.31 mg/L (range of 0.06–0.98 mg/L) to 0.08 mg/L (0.01–0.22 mg/L). Other activated sludge municipal sewage treatment systems in Ontario removed 77% to 100% of the copper at initial concentrations of 0.26 to 0.55 mg/L. More advanced treatment processes such as lime precipitation - activated carbon, ferric chloride - activated carbon, or alum-activated carbon removed over 90% of the copper at initial concentrations of 0.7 to 5 mg/L (Cohen 1977). Other treatment processes, e.g. ozonization, removed >99.9% of the copper (Netzer and Beszedits 1979).

In the United States, the Environmental Protection Agency recommended a limit of 1 mg/L for domestic water supplies (EPA 1976, p. 55). The same limit has also been recommended in Australia (Hart 1974, p. 56). In Canada the Department of National Health and Welfare (HWC 1979) recommended a maximum limit of 1 mg/L

based on taste considerations. The minimum detectable concentration for taste was considered to be 2 to 5 mg/L. Other publications set the lower limit for taste detectability at 1 mg/L (Hart 1974, p. 58; EPA 1976).

Considering that (i) the copper requirements of human metabolism are satisfied by a normal diet, (ii) prolonged ingestion of larger copper doses may produce liver damage, and (iii) the surface waters in Canada do not contain more than 0.5 mg/L of copper, it is recommended that the maximum copper concentration in raw public water supplies should not exceed 0.5 mg/L as total copper.

Aquatic Life and Wildlife

The concentration of dissolved copper in water is governed by a number of factors such as the presence of complexing agents, alkalinity, pH and the type of sediments. The Experimental Lakes Area near Kenora in Ontario had a mean of 0.002 mg Cu/L for 102 lakes, while lakes close to the smelters of Sudbury had a range of 0.005 to 0.011 mg Cu/L with lakes more distant containing 0.003 to 0.004 mg Cu/L (Beamish *et al.* 1975). Offshore waters of the upper Great Lakes contained less (95% of the measurements were <0.005 mg Cu/L with a few to 0.007 mg/L) than onshore waters, which had an average as high as 0.024 mg/L (range of 0.002 to 0.060 mg Cu/L) at municipal water intakes. The higher levels were associated with human activity (IJC 1976a, b). (See also section "Natural distribution of copper.")

Copper is an essential element for plants and animals and is a component of many metalloenzymes and respiratory pigments. The stimulation of algae by the addition of traces of copper was demonstrated by the addition of 0.071 mg Cu/L, as $CuSO_4$, in five doses to experimental ponds at monthly intervals from May to September. The average biomass in copper-treated ponds was 41 g/m^3 compared with that of 27 g/m^3 in the control ponds. Chlorophyta and Bacillariophyceae were most affected, their biomass doubling in the treated ponds. Euglenophyta were adversely affected, and the biomass and density decreased by three times that found in the untreated ponds (Januszko 1976).

Copper toxicity varies with the species of plant or animal and depends on factors such as pH, the presence of complexing agents or other metals, and the species of copper present in the water.

Accumulation of Copper by Algae and Aquatic Macrophytes

Copper can and does accumulate in algae to quite high levels. Mierle and Stokes (1976) reported that an initial rapid uptake of copper was sensitive to the pH but not to the calcium concentration of the medium, and the metal was thought to be bound to the cell wall. A slower mechanism, probably transport across the plasmalemma (protoplasm bounding the cell wall) was very sensitive to pH and to the presence or absence of Ca ions. The presence of Ca ions as low as 10 µmol/L (0.4 mg Ca/L) and a low pH greatly reduced the rate of uptake of copper and loss of potassium by the laboratory strain of *Scenedesmus* sp., although water hardness, as such, does not appear to be a significant modifying factor on the effects of copper. Cedeno-Maldonado and Swader (1974) stated that the amount of copper taken up by *Scenedesmus* cell binding sites, which were probably sulphydryl ligands, decreased with decreasing pH. For *Scenedesmus* not from the Sudbury area, there was a break in the pattern of uptake at pH 6.8. At this point, copper accumulated continuously during the treatment period (25 min), but at lower pH values (pH 5.8, 4.8, 3.8) accumulation appeared complete at 2 min. With tolerant *Scenedesmus* from the Sudbury region, over the whole range of pH tested (3.8 to 6.8), only the fast pattern of uptake occurred and less copper was accumulated.

The initial rate of copper uptake by green algae and blue-greens was fast and the quantity of metal in the cells increased 10-fold when cells were exposed to 0.005 mg Cu/L. The uptake rate then decreased and there was a shift in the uptake ≒ excretion equilibrium towards activation of the processes that eliminate copper from the cells. The maximum copper uptake occurred when copper was present in the medium in the form of a complex with a coordinate bond. Uptake from complexes with a coordinate-covalent bond was much lower. Blue-green algae took up more copper than green algae. The different copper complexes produce unequal loss of potassium from algal cells. Less potassium loss occurred in the presence of copper complexes with coordinate-covalent bonds (35% of the control level over 5 days and 60% after 10 to 15 days) than with coordinate bonds, when the potassium content of cells decreased by 80% in the first 30 to 60 min and then remained practically unchanged during the rest of the experiment. Most of the copper was strongly held in the cells. The loss of potassium disturbed cell metabolism (Khobot'yev *et al.* 1976). The loss of potassium from cells poisoned by copper was one of the primary effects recorded by Kamp-Nielsen (1971) when studying *Chlorella pyrenoidosa*.

Schreffler *et al.* (1977) demonstrated an initial rapid uptake of copper by the alga *Vaucheria sessilis* followed by a lower rate. The total amount of absorbed copper increased with increasing pH.

Stokes (1975) reported that tolerant cells accumulated more copper than non-tolerant cells of *Scenedesmus* exposed to the same level of copper, but Foster (1977) found the opposite to be true for *Chlorella vulgaris*. The non-tolerant strain accumulated 5 to 10 times more metal at comparable external copper concentrations than did the tolerant strain. There was very little difference between the two strains for growth parameters, e.g. lag growth period (a phase in growth, a pause before growth begins, extended by the addition of copper) and yield, at equivalent cellular copper concentrations. It was suggested that copper exclusion was likely to be the only mechanism of tolerance in this alga, thus allowing it to live at higher levels of external copper. Some of the observed effects of copper toxicity were the appearance of giant cells reflecting an uncoupling of growth from cell division. Also, dividing cells failed to separate, resulting in multicellular aggregates that persisted until the late log phase of growth.

The uptake of copper by *Anabaena* 7120, a blue-green; *Ankistrodesmus braunii*, a green alga; and sediment was studied by Laube *et al.* (1979). The sediment was composed of poorly crystallized kaolite and illite with a mean grain size of 0.35 mm and 18.58 meq/100g (dry wt) cation exchange capacity. Experiments were carried out in dialysis sacs suspended in Ottawa River water. Copper was added to the river water (1.02 mg Cu/L as $Cu(NO_3)_2$) and it was taken up by the algae and sediment exposed in separate sacs. Equilibrium was reached between the copper ion-fortified water and the algae in 12 h in a ratio (copper ion accumulated by alga to the copper ion accumulated by the sediment) of 0.65 for *Anabaena* and 1.02 for *A. braunii*. When the sediment was the source of copper, the algae also absorbed the copper. It was suggested that in natural systems the copper is not permanently bound to the sediment and that the small amount released to the water was immediately taken up by the algae, thus equilibrium between sediment and water was not reached. The algae thus may play a role in mobilizing sediment-bound heavy metal ions. Animals grazing on the algae would in turn take up the heavy metal.

Cladophora glomerata (green alga) accumulated copper from filtered Lake Ontario water by factors ranging from 1900 to 2000. Factors of 1000 were obtained for western Lake Erie and 2500 for Spokane River (Keeney *et al.* 1976).

Periphyton exposed to 0.009 and 0.002 mg Cu/L accumulated 159 and 42 mg Cu/kg, respectively. There was a significant positive correlation between the amount of copper in the water and that accumulated by the periphyton. Macrophytes exposed to the same concentrations of copper accumulated 20 to 88 mg Cu/kg in the roots when exposed to 0.009 mg Cu/L, and 6 to 34 mg/kg when exposed to 0.002 mg/L. The leaves accumulated 18 to 81 mg/kg and 4 to 21 mg/kg, respectively. Sediment copper concentrations correlated significantly with copper in the roots of *Eleocharis* and *Nymphaea* but not for the roots of *Equisetum* and *Nuphar* (Hutchinson *et al.* 1975).

High levels of copper (345 mg/kg dry wt) were present in sediments in some parts of the Moira River, Ontario, which drains an area where mining for a number of minerals has been active for over 100 years. Levels of copper in the water from the same areas of the river were low (0.001 to 0.003 mg/L). Macrophytes took up copper in varying degrees, the highest levels being in *Myriophyllum verticillatum* (37 mg/kg) and *Elodea canadensis* (25 mg/kg) in May. Later in the year, levels were lower, probably owing to dilution by an increase in plant biomass. There was a correlation between total copper in the sediments and in the two macrophytes, but not between the extractable copper (0.5 mol HCl/L) in the sediments and the total copper in the plants. Of the plants surveyed, the submerged plants took up more copper than the emergent macrophytes (Mudroch and Capobianco 1979).

Dietz (1973) reported average enrichment factors for aquatic macrophytes growing in water containing 0.01 to 0.1 mg Cu/L of 78 for *Nuphar luteum* with floating leaves, 870 for the submerged *Myriophyllum spicatum* and 2800 for the moss *Hygroamblystegium* sp.

Intracellular Distribution of Copper

The distribution of copper in cells of *Scenedesmus* sp. was examined with the electron microscope. It was suggested that intranuclear inclusions in copper-treated cells were a detoxifying mechanism in which copper is complexed by protein ligands, thus protecting cytoplasmic organelles to a certain extent from the toxic effects of copper. Cellular damage was observed in cells treated with high levels of copper (0.1 mg/L for non-tolerant and 0.7 mg Cu/L for tolerant cells) (Silverberg *et al.* 1976).

Toxicity of Copper to Algae

The distribution of algae in rivers affected by mining activities showed that many algae have been eliminated from areas where the metal levels are high but some exhibited tolerance. For example, *Microspora* spp. and *Ulothrix* spp. (Chlorophyceae) tended to be relatively resistant, but *Oedogonium* spp. were much more sensitive. The morphology of the algae may also have an effect on the susceptibility of the organisms to copper. Two species

forming compact colonies, *Sporotetras pyriformis* and *Gongrosira* sp., where the binding of copper near the outside might be expected to protect cells further inside the colony, were the most resistant of the 25 species tested: levels above 0.57 and 0.84 mg Cu/L, respectively, would be growth inhibitory. The growth of the more sensitive species (*Oedogonium* spp.), which were taken from a number of habitats, were inhibited by 0.12 to 0.29 mg Cu/L, and *Cladophora glomerata* by 0.19 mg Cu/L (Whitton 1970).

McLean and Jones (1975) studied the distribution of the flora in a number of Welsh rivers that had been affected by mining. The alga *Hormidium rivulare* was found throughout the whole range of heavy metal concentrations, but most of the other filamentous algae, for example, *Spirogyra* spp., *Zygnemales* spp. and *Microspora* spp., were more restricted in their distribution. *Ulothrix* spp. are algae that were shown to be relatively resistant to heavy metals, agreeing with findings of Whitton (1970). Bryophytes (mosses and liverworts) were found in relatively pollution free streams, but *Scapania undulata* was also found below the confluences of mine drainage streams (0.02 mg Cu/L) and contained high levels of the metal (e.g. 300 to 500 mg Cu/kg). *Scapania* was shown to accumulate less of the heavy metal than other species of bryophyte, e.g. *Fontinalis squamosa*, when exposed to the same levels of heavy metals in water. *Scapania* also exhibited tolerance to copper and other heavy metals on the North American continent (Shacklette 1965 *in* McLean and Jones 1975).

Stigeoclonium tenue was found in rivers polluted by copper up to a level of 0.02 mg Cu/L. Other metals such as zinc and lead were also present, so that the toxic effect of copper on the alga was not acting in isolation. *Cladophora glomerata* also tolerated a similar level of copper. The upper limit of copper in the survey of the rivers was 0.04 mg Cu/L (McLean 1974). It is possible that growth conditions in the river were not optimum, since other studies have shown that the two algae could tolerate higher levels of copper, especially when *S. tenue* was in the presence of organic pollution to which it is quite tolerant. The formation of copper chelates could also have been a detoxifying factor, as the copper would not be biologically available. The tolerance of *Stigeoclonium* and *Cladophora* did not vary between sample sites (Whitton 1970).

Algae from lakes in the Sudbury region, which are affected by smelter fallout and tailings runoff, are more tolerant to copper than algal strains from areas where the copper content of the water was very low. Algae survived in lakes which had copper levels up to 0.7 mg Cu/L but were absent from those where copper levels were higher. Tolerant algae, e.g. *Scenedesmus* sp. and *Chlorella* sp., isolated from these lakes grew in the laboratory in water with 1.5 mg Cu/L in contrast with non-tolerant algae, which were inhibited by 0.1 mg Cu/L. The tolerant cells accumulated much more copper (1238 mg/kg dry wt) than the others (187 mg/kg dry wt) when grown in water containing 0.05 mg Cu/L, showing that the tolerance to copper was not due to the exclusion of copper by the cells (Stokes 1975). The toxicity and uptake of copper decreased in the presence of chelators in water because complexed copper is not toxic to the algae. The decrease in copper toxicity matched the complexing capacity of the water fairly well (Stokes and Hutchinson 1976).

Effect of Copper on Photosynthesis, Respiration and Growth

Growth of the diatom *Nitzschia lineeris*, measured by comparing the number of cells produced at the end of a 5-day growth period, was reduced to 50% of the control growth by 0.795 to 0.815 mg Cu^{2+}/L. This was considered to be an end point equivalent to the 96-h LC_{50} for fish (Patrick *et al.* 1968).

Growth of *Chlorella sorokiniana* in cultures was slowed by the addition of 1 µmol/L $CuSO_4$ (0.064 mg Cu/L) and completely inhibited by 2 µmol/L $CuSO_4$ (0.127 mg Cu/L). Photosynthesis inhibition occurred within 5 min of the addition to cupric ions exceeding 50 µmol/L $CuSO_4$. At 100 µmol/L, the evolution of oxygen was not inhibited completely, but concentrations exceeding 200 µmol/L were 100% inhibitory. Respiration was not affected at concentrations which reduced growth and photosynthesis, and approximately twice as much copper was needed to give a measurable inhibition of respiration. The effect of copper was less in more dense cultures, probably as there were more cell wall surfaces and non-inhibitory sites to remove copper from the medium than in cultures where there were fewer cells per litre. The effect of the cupric ion was greater under anaerobic conditions than under aerobic conditions. Cupric ions were shown to bind to SH-groups (Cadeno-Maldonado and Swader 1974).

Aphanizomenon sp. and *Anabaena* sp. (blue-green algae) photosynthesis was affected by 0.01 mg Cu/L for the first 3 days of incubation. No additional inhibition was noted after 5 days and then there was evidence of some recovery, so the depression of growth by this level of copper in the natural environment may not be significant. At 0.04 mg/L there were no signs of recovery. Study of a lake community indicated that additions of 0.005 and 0.01 mg/L did not inhibit total accumulation of

algal biomass but did cause a shift in community structure resulting in the near elimination of blue-green genera and an increase in green algae and diatoms (Elder and Horne 1978). Nitrogen fixation was also affected by trace amounts of copper (0.005 to 0.01 mg/L) added to a lake with natural copper levels ranging from undetectable to 0.033 mg/L (means in April and September of 0.0037 and 0.0027 mg/L, respectively). The added amounts were approximately 1/200 of the copper concentration used for algal control. Addition of 0.005 mg Cu/L reduced nitrogen fixation by 76% in 2 days and by 90% to 95% after the fourth day of the experiment (8 to 10 days, laboratory incubation at 20°C). Photosynthesis was reduced by only 7% to 34%. The addition of 0.01 mg Cu/L caused a permanent drop in nitrogen fixation, which reached zero in 2 days. Addition of 0.005 and 0.01 mg Cu/L affected the blue-green *Anabaena* less than *Aphanizomenon* but did not affect the diatom *Melosira* or the green alga *Oocystis*. Addition of copper to lakes (e.g. Clear Lake) has been shown to cause changes in the dominant blue-greens, indicating different sensitivities in the blue-greens. Lake copper was presumably chelated and the existence of excess chelating capacity was suspected, thus no changes in toxicity were apparent with the fluctuating levels of lake copper (Horne and Goldman 1974).

Copper affected photosynthesis in *Chlorella pyrenoidosa* (Chlorophyta) and *Nitzschia palea* (Bacillariophyceae) differently. Copper did not penetrate the cells of *C. pyrenoidosa* immediately but influenced the alga by blocking mechanisms in the cell membrane so that no cell division took place. It seemed that the decline in the rate of photosynthesis a number of hours after exposure to copper was due to the accumulation of photosynthetic products which secondarily blocked photosynthesis. After 4 h, a decrease in photosynthesis was only observed at high light intensities with a high photosynthetic rate. With *Nitzschia palea* there was a decrease in the photosynthetic rate after 20 min, and the percentage decrease was similar at all light intensities. The copper probably penetrated the diatom cell. The cells lost up to 22% of the synthesized organic matter, indicating that the cell membrane had been altered in some way. Concentrations as low as 0.001 to 0.002 mg Cu^{2+}/L were shown to suppress photosynthesis in *N. palea*. Effects on growth were noted at 0.005 mg/L for both species. Copper was found as a contaminant in distilled water used in ^{14}C measurements of photosynthesis, and thus many experiments using this technique may have been affected by an unsuspected toxic effect. (Steemann Nielsen and Wium-Andersen 1970). It was proposed that at least some of the metal ions were bound to this organic matter, thus removing part of the toxic effect of the copper (Steemann Nielsen and Wium-Andersen 1971). Swallow *et al.* (1978) reported that extracellular organic compounds produced by *Gloeocystis gigas* (Chlorophyceae) measurably reduced cupric ion toxicity in the culture medium. The medium was sterile, thus eliminating the possible complexing action of microorganisms. The other algal species tested (*Thalassiosira pseudonana* (marine), *Ankistrodesmus falcatus*, *Pediastrum* sp., *Staurastrum gracile*, *Tribonema* sp., *Cyclotella cryptica*, *Chlamydomonas* sp.) also exuded organic material but this did not complex with the copper so as to decrease the copper toxicity. Van den Berg *et al.* (1979) have shown that algae exude materials which are capable of complexing with copper and thus reducing its toxicity towards primary production in *Chlorella vulgaris* (green alga). The algal species used to obtain complexing ligands were *Anabaena cylindrica*, *Scenedesmus quadricauda* and *Navicula pelliculosa* grown in CHU-10 medium. The ligands were produced in varying quantities and with varying complexing capacity. *Anabaena* filtrate had the highest complexing capacity (6.73 µmol/L) and protected *Chlorella* against copper toxicity up to 0.2 mg Cu/L, the highest concentration tested. The primary production of *Chlorella* was decreased by 8%, owing to copper toxicity, in the presence of *Anabaena* filtrate, but the effect of copper was not ameliorated as much by the other two filtrates. There was a 45% decrease in primary production owing to copper in *Navicula* filtrate and 36% decrease in *Scenedesmus* filtrate. Much more work is needed to determine whether this type of complexation of copper is a significant factor in decreasing the toxic effect on algae in the natural situation. The effect of algal excretory products decreasing the toxicity of copper to algae was also shown by Fogg and Westlake (1955), and self-produced organic molecules were shown to reduce metal toxicity to phytoplankton (Huntsman and Barber 1975).

Ten days after 0.0005 mg Cu/L (as $CuCl_2$) was added to cultures of *Scenedesmus quadricauda* (green alga) and *Anabaena variabilis* (blue-green) the number of individuals had decreased by 1/3 and 1/10 to 1/15 of the control numbers, respectively. During the next 20 days the growth of the blue-green alga was still suppressed, but *Scenedesmus* had mostly overcome the effects of the copper and its numbers had doubled over the initial density of the inoculum (Khobot'yev *et al.* 1975). This difference in sensitivities between algal groups had been demonstrated by other authors (Steemann Nielsen and Laursen 1976).

Effects of pH on Copper Toxicity—Algae

Increasing tolerance to copper was found with increasing pH (4.0 to 7.2) when *Chlorella ellipsoidea* was grown in an artificial medium in the presence of 20 mg Cu/L (Kanazawa and Kanazawa 1969). The con-

verse was found by Steemann Nielsen and Kamp-Nielsen (1970), as the tolerance of *Chlorella pyrenoidosa* was greater at pH 5 than at pH 8. The uptake of copper was also less at pH 5 (Steemann Nielsen *et al.* 1969). The toxicity of copper to the alga *Hormidium rivulare* also increased markedly at pH values above 3.5 to 4.0. This alga inhabits streams affected by acid mine drainage and has an optimum growth at pH 3.5 to 4.0 (Hargreaves and Whitton 1976).

The density and taxonomic composition of the algae and the presence of humic compounds and pH of the water had an effect on the toxicity of copper ions to phytoplankton. The blue-green *Microcystis* sp. was shown to be very sensitive to copper (Steemann Nielsen and Laursen 1976) and this agreed with the findings of Elder and Horne (1978) and Khobot'yev *et al.* (1975).

The results of studies by different authors are not directly comparable because of different experimental conditions, such as different culture media. Thus, their use as criteria for the determination of water quality objectives is limited.

Effects of Mixtures of Contaminants—Plants

Pollutants rarely occur in isolation and the interaction of copper with other contaminants should always be considered very carefully when determining detrimental effects in the natural environment. When more than one metal is present, the effects are rarely simply additive.

Wong *et al.* (1978) studied the effects of mixtures of metals on the primary productivity and cell numbers of four species of algae: *Scenedesmus quadricauda* and *Chlorella pyrenoidosa* (green algae), *Anabaena flos-aquae* (blue-green) and a diatom, *Navicula pelliculosa*. Two test media were used: Hamilton Harbour water, which is a polluted and eutrophic body of water, and CHU-10 medium. Both had similar pH and hardness. The initial concentrations in the solution of metals were sodium arsenate, 0.05 mg/L; cadmium nitrate, 0.0002 mg/L; potassium dichromate, 0.05 mg/L; copper sulphate, 0.005 mg/L; ferrous sulphate, 0.3 mg/L; lead nitrate, 0.025 mg/L; mercury chloride, 0.0002 mg/L; nickel sulphate, 0.025 mg/L; sodium selenite, 0.01 mg/L; and zinc chloride, 0.03 mg/L. None of the metals were toxic at these concentrations when they were alone. When the mixture of metals formed 10% of the medium, the primary productivity of *S. quadricauda* decreased to 60% of that in the CHU-10 medium and 79% of that in Hamilton Harbour water without added metals. In a mixture made up of 50% CHU-10 and 50% metals solution, the primary productivity further decreased to 58% and 31% of the productivity in the controls, CHU-10 and Hamilton Harbour water, respectively. The algal species varied in their response to the toxicity: the diatom was the most sensitive, followed by blue-green and green algae. In most natural waters, metals are present together, not alone, and when mixed together may have synergistic or antagonistic effects. The CHU-10 medium represented an unpolluted lake water, and Hamilton water already contained low concentrations of various metals, which had an additional toxic effect on the algae.

Nickel and copper act more than additively in their toxic action for a number of algae. Nickel alone was not as toxic as copper, but when the two metals were together, a synergistic effect was present. The growth of *Scenedesmus* sp. was reduced by 40% of the control growth by 0.3 mg Cu/L and 22%, by 1.0 mg Ni/L, but when the two metals were together at the same concentrations, the effect was more than additive and growth was inhibited by 75% of the control growth (Stokes and Hutchinson 1976). Larger amounts of copper and nickel were also taken up by algal cells when the two metals were together in solution than when they were alone: 2366 mg Cu/kg dry weight was taken up in 6 days in the presence of 0.3 mg Cu/L alone; when 3.0 mg Ni/L was also present, 4177 mg Cu/kg algal dry weight was taken up. More nickel was taken up by the cells in the presence of copper (Stokes 1975).

The presence of nickel and copper accentuated the toxicity of either metal for *Lemna valdiviana*. In a medium containing 0.3 mg Cu/L, the mean growth of 41 leaves occurred, and in 0.3 mg Ni/L, 435 leaves were produced. When 0.3 mg/L of each metal was present together, there was no growth and death occurred. Both copper and nickel uptakes were increased by the presence of the other metal (Hutchinson and Czyrska 1975).

Copper in soft water (14.9 mg/L as $CaCO_3$) was algicidal to *Selenastrum capricornutum* (green alga) at 0.3 mg/L, and growth inhibition was initiated by 0.05 mg/L. Complete inhibition of growth was caused by 0.09 mg/L. The most noticeable effect on growth rate was a 24-h extension of the lag growth phase caused by 0.05 and 0.06 mg Cu/L; 0.07 mg/L caused a 48-h extension and 0.08 mg/L, a 144-h extension. Copper and cadmium combinations resulted in greater growth than that which occurred when the same concentrations of copper were present alone and suggested that cadmium inhibited the toxicity of copper (Bartlett *et al.* 1974).

Silver was shown to enhance the toxicity of copper to phytoplankton (White and Lisk 1970).

Iron has been shown to depress the absorption of copper by some plants, e.g. rice plants, but not others. Copper affected the absorption of iron by widely different rates depending on the test plant. The apparent toxic effect of copper to some algae (and terrestrial plants) has been suggested to be due to the inhibition of iron uptake, which causes an iron deficiency, and not due to the direct toxic effect of copper (Dokiya *et al.* 1968).

The presence of a chelator such as EDTA (ethylene diaminetetraacetic acid) (0.05 mg/L) decreased the uptake of copper and this was paralleled by a decrease in toxicity to *Scenedesmus* sp. taken from a contaminated lake. Cells, in the presence of 0.1 mg Cu/L and no EDTA, took up approximately 0.025 mg of copper per cell culture. In the presence of a higher concentration of copper (0.8 mg Cu/L) and 0.05 mg EDTA/L, the cells took up much less copper (<0.004 mg Cu). The differing complexing capacity of several lakes near Sudbury was shown to have different effects on copper toxicity. In lakes with negligible complexing capacity, algal growth was reduced by 20% in the presence of 0.2 mg Cu/L. In lake water with high complexing capacity, algal growth was not affected by 0.2 mg Cu/L (Stokes and Hutchinson 1976). In a preliminary report (ERL-D 1976), NTA (nitrilotriacetic acid) chelates protected algae and other aquatic organisms from the toxicity of 0.1 and 0.03 mg Cu/L.

The presence of copper as a trace element was shown to affect the toxicity of paraquat (1,1'-dimethyl-4,4'-bipyridinium, 2.0 mg/L) by inhibiting chlorosis in *Chlorella pyrenoidosa* (green alga). It was thought that copper-containing enzymes played some role in decreasing the paraquat phytoxicity (Tsay *et al.* 1970).

The heterotrophic activity of microflora was inhibited by the addition of 0.01 mg Cu/L. In combination with zinc (0.05 mg/L) the toxicity of both metals was approximately the same as the sum of the individual metals, indicating an additive effect. The numbers of viable heterotrophic bacteria were not altered by the above levels of heavy metal (Albright and Wilson 1974).

Macrophytes

The growth of duckweed (*Lemna valdiviana*), a floating vascular plant, varied inversely with the amount of copper accumulated in the tissues. The yield in 0.1 mg Cu/L was half that at 0.01 mg Cu/L. The copper concentration in the plant was greater than 10 000 times the concentration in the culture solution and the concentration factor appeared to increase at the higher levels of metal in solution. Above 0.1 mg/L, the copper entered the cells very quickly and caused severe growth inhibition and death, suggesting that the adverse effect was on the cell membrane (Hutchinson and Czyrska 1975).

Nuphar variegatum and *Potamogeton* sp. growing in Indian Lake, Massachusetts, were not visibly affected by 0.116 mg Cu/L (Symmes 1975).

Accumulation of Copper by Macroinvertebrates

Concentration factors (concentration in tissues/concentration in water) for aquatic invertebrates range quite widely (500 to 13 500); examples are given in a report by Spear and Pierce (1979b, p. 46). A few are discussed in the following paragraphs.

Copper-tolerant *Asellus meridianus* (crustacean) accumulated copper from water and food in concentrations that proved lethal to non-tolerant populations. The studies suggested that the hepatopancreas was important in the storage of copper. *Asellus* from tolerant and non-tolerant populations were exposed to 0.5 mg Cu/L for 8 days and at the end of the exposure, the tolerant populations had accumulated 1170 mg Cu/kg compared with 590 and 430 mg/kg (dry wt) by non-tolerant animals. When the hepatopancreas and intestine were removed, the differences were much less, 490 mg/kg (tolerant) compared with 348 and 370 mg/kg (dry wt), respectively. The tolerant animals were more capable of detoxifying and storing the copper than animals from rivers that were not contaminated by copper (Brown 1977a).

Various organisms living in a system contaminated by coal ash accumulated copper by differing amounts. The copper concentration in the water was 0.40 mg/L; the sediment, 32 mg/kg; rooted and floating macrophytes and a family of blue-green algae, 14 mg/kg; macroinvertebrates, 67 mg/kg; and the mosquito fish and tadpoles (*Rana* sp.), 38 mg/kg (wet wt) (Cherry and Guthrie 1977).

The accumulation of copper differed for a number of insects. Exposed to a level of 0.3 mg/L soluble copper in water at a sampling site, Trichoptera (caddisflies) accumulated 1000 mg/kg; Plecoptera (stoneflies), 615 mg/kg; Odonata (dragonflies), 768 mg/kg; Neuroptera (spongilla flies), 183 mg/kg; and adult Coleoptera (beetles), 47 mg/kg (dry wt), which indicated that there was a wide range in the amount of copper that can be accumulated by insects. Increases in the amount of copper in the tissues of non-case-bearing Trichoptera correlated with the increase in copper in the water but did not do so for Plecoptera and Odonata. There was no significant correlation between the total copper in either the sediment or water and the concentration in the invertebrates (Brown 1977b).

The mean concentrations of copper in clams and in tubificids (worms) taken from sediments and water containing 19 mg Cu/kg and 0.001 mg Cu/L, respectively, were 1.2 to 1.7 mg/kg for the clams and 10 to 42 mg/kg (wet wt) for the tubificids (Mathis and Cummings 1973).

Toxicity of Copper to Invertebrates

Studies with *Daphnia magna* showed that the toxicity of copper was due to the cupric ion activity. Water had an effect on copper toxicity through changes in ionic activity either by precipitation or by complex formation. None of the soluble complexes or chelated forms were appreciably toxic. Calcium and magnesium did not appear to be major factors in affecting copper toxicity. Both bicarbonate alkalinity and orthophosphate played major roles in limiting copper toxicity in natural waters. *Daphnia* survival was extended from approximately 65 min to over 3 days by the addition of the equivalent of 1.5 mg total P/L to solutions containing 0.318 mg total Cu/L. The cupric ion was also the toxic species with fathead minnows. Very similar LC_{50}'s (approximately 0.06 μmol Cu^{2+}/L) based on cupric ion activity were obtained in two types of water with different complexing capacities. The total copper concentrations were very different from the two waters: 0.2 and 0.8 mg total Cu/L (Andrew 1976). The toxicity of copper to *Daphnia magna* was decreased by the addition of bicarbonate anions, which complexed with the copper (Andrew *et al.* 1977).

The toxicity of copper to *Daphnia magna* in Lake Superior water (hardness 45 mg/L as $CaCO_3$) was tested by Biesinger and Christensen (1972). The presence of organic food material affected the toxicity of copper. The 3-week study of mortality and copper effects on reproduction involved feeding. The 3-week LC_{50} was 0.044 mg Cu^{2+}/L, the concentration causing 50% reproductive impairment was 0.035 mg/L and that causing 16% reproductive impairment was 0.022 mg/L. The 48-h LC_{50}, also with food, was 0.06 mg Cu^{2+}/L, but without food copper was much more toxic, 0.0098 mg Cu^{2+}/L. In the field the latter situation would be unrealistic, but inadequacy of food would have an effect on copper toxicity.

The effect of food on the toxicity of copper to *Daphnia magna* was also studied by Winner *et al.* (1977). The two types of food, algae and trout granules, did not appear to affect acute toxicity, as the 72-h LC_{50} in five experiments using the two types of food ranged between 0.0814 and 0.0888 mg Cu/L. The concentrations of the toxic copper species were the same with the two diets, since the LC_{50}'s were not significantly different and the concentrations of soluble copper measured in the two culture media were not significantly different. The longevity, reproduction and instantaneous rate of population growth measurements indicated that *Daphnia* which had been fed trout granules were more sensitive to copper over a longer time period and it was suspected that a nutritional inadequacy was the cause. The algal-fed *Daphnia* lived much longer than those fed on trout granules. Differences occurred in the chronic studies and it was suggested that the modes of action in the two types of toxicity (acute and chronic) were different and that environmental factors could have wide-ranging effects, making the extrapolation of laboratory studies to the field very difficult if an application factor (AF) was used. The AF from algal-fed *Daphnia* was 0.45 to 0.49 and for *Daphnia* fed trout granules, 0.12, derived from the 72-h LC_{50}; MATC's (maximum acceptable toxicant concentration) were 0.04 mg/L and 0.01 mg Cu/L for algal-fed *Daphnia* and *Daphnia* fed trout granules, respectively.

Four species of *Daphnia* differed in the response to acute copper exposure. The 72-h LC_{50} for *D. magna* and *D. pulex* was 0.086 mg/L; for *D. parvula*, 0.072 mg/L; and for *D. ambigua*, 0.0677 mg/L. The length of survival in days was significantly reduced for all species at concentrations >0.04 mg Cu/L. Reproductive sensitivity was more variable. *Daphnia magna* was least sensitive to chronic copper stress; it was the only species where copper stimulated reproduction and there was no significant reduction of reproductive success, exhibited by smaller brood size, at any of the copper concentrations tested (up to 0.1 mg Cu/L). Reproduction was inhibited only because females died before they reached reproductive maturity. Mean brood size of *Daphnia pulex* was significantly reduced at 0.08 mg Cu/L and that of *D. parvula* at 0.06 mg Cu/L. *Daphnia ambigua* was the most sensitive, and broods were all significantly smaller when exposed to 0.06 mg/L. A concentration of 0.04 mg/L also appeared to have the same effect. Using longevity as the least variable parameter, rather than brood size as a measurement of sensitivity to chronic copper stress, there were no significant differences between the four species (Winner and Farrell 1976).

Chelates of copper with NTA (nitrilotriacetic acid) were relatively nontoxic, and appeared to be toxic only when copper was present in excess nearly equal to or greater than the LC_{50} of the metal alone. The adult 3-week LC_{50} for *Daphnia magna* exposed in Lake Superior water (hardness 45 mg/L as $CaCO_3$) to copper alone, was 0.044 mg/L and for copper with 2.0 mg Na_3NTA/L, 0.26 mg Cu/L. Fifty per cent reproductive impairment was caused by 0.035 mg Cu/L, and when complexed with NTA, 0.26 mg Cu/L was needed to produce the same result (Biesinger *et al.* 1974). Short-term toxicity data are also given in Table 3 (amended from Spear and Pierce 1979b).

Table 3. The Short-term Lethal Tolerance of Freshwater Invertebrates (taken from Spear and Pierce 1979b)

Organism	Temperature (°C)	pH	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	LC_{50} (mg/L)	Reference
Gammarus pseudolimnaeus Crustacea	-	7.7	45	43	0.02 (96-h)	Arthur and Leonard 1970
Limnodrilus hoffmeisteri Oligochaeta	22	7.8	100	60	0.100 (96-h)	Wurtz and Bridges 1961
Biomphalaria glabrata Gastropoda	(deionized water)				3.2 (24-h)	Sullivan and Cheng 1975
Acroneuria lycorias Insecta	18	7.3	44	40	8.3 (96-h)	Warnick and Bell 1969
Ephemerella subvaria Insecta	18	7.3	44	40	0.32 (96-h)	Warnick and Bell 1969
Philodina acuticornis Rotifera	20	7.9	25	24	0.7 (96-h)	Buikema *et al.* 1974
Philodina acuticornis Rotifera	20	7.4-7.8	81	54-67	1.1 (96-h)	Buikema *et al.* 1974
Campeloma decisum Gastropoda	-	7.7	45	43	1.7 (96-h)	Arthur and Leonard 1970
Physa integra Gastropoda	-	7.7	45	43	0.039 (96-h)	Arthur and Leonard 1970
Physa sp. Gastropoda	-	7.2	(25?)	10	0.035*	Howard *et al.* 1964
Physa sp. Gastropoda	-	7.8	(160?)	130	0.083*	Howard *et al.* 1964
Gyraulus circumstriatus Gastropoda	22	7.8	100	60	0.11*	Wurtz and Bridges 1961
Physa heterostropha Gastropoda (pre-adult)	22	7.3	20	-	0.016*	Wurtz 1962
Physa heterostropha Gastropoda (pre-adult)	22	7.8	100	-	0.013*	Wurtz 1962
Physa heterostropha Gastropoda (adults)	22	7.8	100	-	0.069*	Wurtz 1962
Goniobasis livescens Gastropoda	-	-	150	-	0.86 (48-h)	Cairns *et al.* 1976
Lymnaea emarginata Gastropoda	-	-	150	-	0.3 (48-h)	Cairns *et al.* 1976
Daphnia magna Crustacea	18	7.4-8.2	45	42	0.06 (48-h)	Biesinger and Christensen 1972
Daphnia magna	20	8.2-9.5	130-160	100-119	0.08-0.085 (72-h)	Winner *et al.* 1977
Daphnia magna	20	8.2-9.5	130-160	100-119	0.0865 (72-h)	Winner and Farrell 1976
Daphnia pulex	20	8.2-9.5	130-160	100-119	0.086 (72-h)	Winner and Farrell 1976
Daphnia parvula	20	8.2-9.5	130-160	100-119	0.072 (72-h)	Winner and Farrell 1976
Daphnia ambigua	20	8.2-9.5	130-160	100-119	0.0677 (72-h)	Winner and Farrell 1976

*Exposure time less than 96 h.

The toxicity of copper to the protozoan *Paramecium tetraurelia* increased linearly as the temperature increased over the range of 12°C to 34°C. The 24-h LC_{50}'s at 12°C, 20°C, 27°C and 34°C were 11.7, 10.4, 6.9 and 4.7 μmol Cu^{2+}/L respectively (0.743, 0.661, 0.438 and 0.299 mg Cu^{2+}/L) (Szeto and Nyberg 1979).

Miles *et al.* (1976) tested four invertebrates which had been pre-acclimated to a number of test temperatures (10°C, 15°C and 20°C). The sensitivity to copper increased as temperature increased. The 24-h LC_{50} for *Daphnia pulex* at 10°C, 15°C and 20°C was 0.07, 0.03 and 0.02 mg Cu/L respectively; for *D. magna*, 0.09, 0.05 and 0.01 mg/L; for the rotifer *Philodina acuticornis*, 0.43, 0.3 and 0.21 mg Cu/L; and for the snail *Nitocris* sp., which was the least sensitive organism of the four, 3.2, 1.8 and 0.56 mg/L, respectively.

The asexual budding of *Hydra* was used to determine the no-effect level of copper for the freshwater coelenterate cultured in a synthetic medium. *Hydra* counteracted the inhibitory effects of stress on reproduction by an increase in the budding rate in an oscillatory manner, and if the stress was not too great, after a short interval the number of buds was not significantly different from the controls and the rate became similar. At 0.005 mg Cu/L and above, the capacity of *Hydra* to counteract the inhibitory effect was overcome, so that after the initial oscillations at 0.005 and 0.01 mg/L the rate of reproduction declined with increase in concentration. At 0.0005 and 0.0025 mg Cu^{2+}/L, there was a marked effect on the budding rate in the early days of the 11-day experiment, but the counter-response restored the rate to that of the unstressed controls. The sensitivity of *Hydra* apparently increased with time and towards the end of the experiment (11 days) the curve became asymptotic, indicating that no further increase in sensitivity would occur. The level of no effect lay between 0.0025 and 0.005 mg/L (Stebbing and Pomroy 1978).

The 48-h LC_{50}'s for *Tubifex tubifex* (oligochaete) of 0.21 mg/L and 0.89 mg/L were obtained in water of hardness 34.2 and 261 mg/L as $CaCO_3$, respectively (Brkovic-Popovic and Popovic 1977).

Loss of osmotic control owing to copper was demonstrated by Cheng and Sullivan (1977) for the snail *Biomphalaria glabrata* (Pulmonata: Gastropoda). The wet weight of snails exposed to 0.06 mg Cu/L as $CuSO_4$ increased as a function of time, but that of the controls decreased. The experimental periods were 24 and 48 h without food. The dry weights of both sets of snails decreased similarly, so that the change in wet weight was caused by an accumulation of water in the tissues of the exposed snails. The osmolality of the hemolymph of the exposed snails decreased and this was thought to be due primarily to dilution by water. The hemolymph of the snails was hyperosmotic to the surrounding water. The exact mechanism of the change in osmoregulation was not understood and needed further study (see also under "Osmoregulation").

Average 96-h LC_{50} values for total copper were 1.7, 0.039 and 0.02 mg/L for *Campeloma decisum* (mollusc), *Physa integra* (mollusc) and *Gammarus pseudolimnaeus* (crustacean), respectively. Long-term tests included growth, percentage survival of the snails and also the number of young produced by *Gammarus*. The test water was from Lake Superior (hardness of 45 mg/L as $CaCO_3$). No growth was observed for *Physa* or *Gammarus* above 0.008 mg Cu/L. Growth was not measurable in *Campeloma* and the snail refused food in copper concentrations above 0.008 mg/L. The 96-h LC_{50} for *Physa* and *Campeloma* (Mollusca) were very different, but after an exposure of 6 weeks, the no effect concentrations were the same. The snail *Campeloma* could seal itself off by means of the operculum, so that probably opercular snails possess a defence mechanism which could be used providing that exposure was not too long and therefore they could survive when other snails such as *Physa* would be killed. Newly hatched *Gammarus* did not grow to adult size in copper concentrations above 0.0046 mg/L. There was a 60% survival at 0.0046 mg/L, 65% at 0.0029 mg/L and 80% at 0.002 mg/L (Arthur and Leonard 1970).

The 48-h LC_{50} for *Gammarus fasciatus* in hard water (206 mg/L as $CaCO_3$) was given as 0.19 mg Cu/L (Judy 1979), similar to that for rainbow trout (*Salmo gairdneri*) of 0.15 to 0.2 mg Cu/L in water with a hardness of 125 to 175 mg/L as $CaCO_3$ (Geckler *et al.* 1976).

Hubschman (1967) reported that copper at levels above 1.0 mg Cu/L rapidly inhibited respiratory enzymes in crayfish. At concentrations below 1.0 mg/L a different toxic action was observed, i.e. there was degeneration of cells and tissues of actively secreting organs, such as the antennal gland, which is concerned with osmoregulation. Cell repair did not keep pace with destruction. Crayfish survived for about 30 days at the lowest concentration tested, 0.5 mg Cu/L. Earlier studies on young and adult crayfish (Hubschman 1965) showed that the young were more sensitive; the acute toxicity threshold for newly hatched young lay between 0.06 and 0.125 mg Cu/L. The 96-h LC_{50} for intermoult adults was 3.0 mg/L. Exposure to copper of 0.015, 0.03 and 0.06 mg/L for 30 days retarded the growth of young crayfish by 28%, 91.4% and 98.6% of the control growth, respectively. The work also showed that mortality occurred long after

the initial exposure, when the crayfish had been removed to clean water. Embryos were much more susceptible than adults; 52% did not survive exposure to 0.25 mg Cu/L over the last 13 or 14 days of development, so that even though egg-carrying females would not succumb, reproduction would be affected by embryos and young dying or by growth of young being severely retarded.

Exposure to 0.8 to 2.4 mg Cu/L increased the oxygen consumption rate of stonefly nymphs (*Pteronarcella capitata*) by 24% to 30% of the control rate. An increase in the physical activity of the nymphs occurred. This increase in activity has also been reported for fish exposed to copper (Kapoor and Griffiths 1976).

Avoidance of Copper—Invertebrates

Fish food organisms (mayflies) were studied to determine whether they would avoid copper to the same extent or more than predatory fish. If the tolerance of food organisms was less, this could affect the nutrition of resident fish. Mayfly nymphs avoided 0.1 mg Cu^{2+}/L, but the avoidance was not significant for 0.01 and 0.001 mg Cu^{2+}/L after 1 h exposure (Folmar 1978). The avoidance level was greater than that for rainbow trout fry, which was 0.0001 mg Cu/L (Folmar 1976). Thus, for this particular food organism, if fish were present and using mayfly nymphs as a major source of food, the supply should be adequate, as there would not be a tendency for the mayflies to avoid or become lost to the area in drift due to copper; the fish would be affected by the copper first.

Avoidance of copper by *Gammarus lacustris* (crustacean) showed that some invertebrates, as with fish, did not avoid all lethal concentrations. Copper concentrations up to 2 times greater (0.19 and 0.46 mg Cu/L) than the 96-h LC_{50} (0.06 and 0.32 mg Cu/L) were avoided, but *Gammarus* was attracted to copper concentrations 40 to 300 times greater than the 96-h LC_{50} (12.3 and 30.0 mg Cu/L). Implications from these types of results are that even though areas free from contamination are present in a body of water, organisms will not necessarily avoid toxic concentrations and, indeed, they may actively seek them out (Maciorowski *et al.* 1977).

Hardness—Invertebrates

Hardness did not appear to have a large effect on copper toxicity to invertebrates, but there was little data that could be directly compared. Toxicity was greater in soft water, e.g. the 96-h LC_{50} for *Philodina rotifera* was 0.7 and 1.1 mg Cu/L in water of hardness 25 and 81 mg/L as $CaCO_3$, respectively (Buikema *et al.* 1974).

Tolerance to Heat after Exposure to Copper

Exposure to copper increased the susceptibility of snails to rapid increases in heat. An application factor of 0.2 was applied to the 48-h LC_{50} for copper for *Goniobasis livescens* (0.86 mg/L) and *Lymnaea emarginata* (0.3 mg/L) to give a sublethal concentration for prior exposure to copper before heat stress was applied. The survival time of *G. livescens* on exposure to heat shock (24°C and 36.5°C) was lessened significantly after exposure to copper (0.17 mg Cu/L, water hardness 137 to 171 mg/L as $CaCO_3$). *Lymnaea emarginata* was significantly affected only if heat shock was given concomitantly with exposure to copper. If the snails were heat shocked after removal to clean laboratory water, the reaction was not significantly different from that of the controls (Cairns *et al.* 1976).

Toxicity of Mixtures of Metals to Invertebrates

The rotifer *Philodina acuticornis* was used to test the interactions of a number of inorganic compounds, using dilution water (hardness 40 mg/L) made up in the laboratory. If the lethal actions were strictly additive, a 50% mortality would be predicted for all combinations of toxicants. There was basically no interaction between the toxicants in mixtures of zinc and copper. Fluoride and copper did not appear to affect the toxicity of each other or they were slightly antagonistic, the mixture being less toxic than expected. The toxicities of copper and chlorine were basically additive, and the mixtures were as toxic as either of the two solutions, copper or chlorine acting alone. Copper and chromium were additive, but there was some variation about the "strictly additive" line of the graph. The results for the rotifer were not always comparable to those for fish and probably reflected different physiological responses. The criterion for no effect on 50% of the animals (EC_{50}) was no visible internal or external motion when exposed to a bright light. Many of the withdrawn rotifers were dead but some were able to recover. The 24-h and 48-h EC_{50}'s were 0.22 and 0.14 mg Cu/L, respectively (Buikema *et al.* 1977).

Field Study—Invertebrates

A study using a natural stream community (Shayler Run, Ohio) experimentally polluted with copper indicated that the number of macroinvertebrate species was the most sensitive of four indices of the impact of copper, though the four (Shannon, Margalef, species numbers, numbers of individuals) indicated the same pattern of community response. The water had a mean alkalinity of 195.5 mg/L, pH near 8.0 and dissolved oxygen always near saturation. The copper concentration just below

the point where copper was introduced, was 0.12 mg/L and it fell to 0.023 mg/L at the last station in the test stretch. The most sensitive groups were *Psephenus* sp. (beetle), *Baetis* sp. and *Stenonema interpunctatum* (mayfly) and *Lirceus* sp. (Isopods, crustacean). These organisms disappeared when the copper concentrations were either 0.12 or 0.066 mg/L. Chironomids (midges) dominated the community when the copper concentrations were 0.12, 0.066 and 0.052 mg/L, the domination decreasing as the copper concentration decreased. Zygopterans (dragonflies) were at their greatest density when the copper concentration was 0.066 mg/L, and a species of *Stenelmis* (beetle) and *Cheumatopsyche* (caddisfly) each attained maximum densities at 0.038 mg Cu/L. The increases in densities of the various groups were probably indirectly related to copper through a lessening of competition and predation, or both. The community composition at the station with 0.023 mg/L was very similar to the control station, which had a mean of 0.009 mg Cu/L (Winner *et al.* 1975).

Toxicity of Copper to Amphibians

Embryo-larval stages of the leopard frog (*Rana pipiens*) were more sensitive to copper than rainbow trout embryos and alevins. The 4-d post-hatch LC_{50}'s were 0.05 mg and 0.11 mg Cu/L, respectively. The 4-d post-hatch for the marbled salamander (*Ambystoma opacum*), gray tree frog (*Hyla chrysoscelis*) and Fowlers toad (*Bufo fowleri*), found in the eastern United States, were 0.77 mg, 27 mg and 0.04 mg Cu/L, respectively (Birge and Black 1979). Amphibian embryos are much more susceptible than adults, e.g. the mature leopard frog survived 4 mg Cu/L for 30 days (Kaplan and Yoh 1961).

Toad embryos and larvae were shown to be as sensitive to copper as those of rainbow trout. The test periods were conducted from fertilization to 4 days post-hatch and involved 28 days for trout and 7 days for the narrow-mouthed toad (*Gastrophryne carolinensis*). The water hardness was 195 mg/L as $CaCO_3$ for the toad and 104 mg/L as $CaCO_3$ for the trout. The test water was renewed every 12 h. The 28-d LC_{50} and LC_1 (1% death) for the trout were 0.09 and 0.0018 mg Cu/L, respectively, and the 7-d LC_{50} and LC_1 for the toad were 0.04 and 0.001 mg Cu/L, respectively. Goldfish were much more tolerant and the 7-d LC_{50} and LC_1 were 5.2 and 0.299 mg Cu/L, respectively (Birge 1978).

Accumulation of Copper in Fish

Green sunfish were exposed to copper in a pond dosed with 3 mg Cu/L as $CuSO_4$. The aqueous concentration declined as the copper accumulated in the mud and remained there for the duration of the 4-month study. After one week the fish had accumulated an equilibrium concentration of 3 mg Cu/kg (wet wt) in their tissues, which decreased to background levels in 70 days. The copper remained in the fish for 5 weeks longer than in the pond water (McIntosh 1975).

The rate of uptake of copper by the gills of juvenile and adult sunfish (*Lepomis gibbosus*) was initially fast, with a slow phase following after approximately 4 h of exposure. Uptake by rainbow trout occurred at only one rate. The uptake rate corresponded with alterations in respiratory activity, which probably resulted from toxicant exposure. The rate of accumulation in sunfish during the slow phase decreased as body weight increased. In general, smaller fish have a larger lamellar surface area to the gills compared to body weight and thus more sites for copper uptake per unit weight. Loss of copper from the gills was monophasic and the rate decreased with increase in body weight. The half life of copper in the fish varied from 1.6 to 4.8 h according to body weight, and fish could survive short periods of exposure to lethal concentrations. Histological examination of gill tissue from copper-exposed fish showed that the cellular damage was quickly repaired on removal from copper solutions. The smaller fish accumulated more copper per unit body weight (Anderson and Spear 1980a). Pollutants are not usually experienced as a continuous exposure, and experiments such as this show that aquatic life can recover from the effects of exposure to lethal levels of toxicants if the exposure is of short duration.

There was very little variation in the copper concentrations (an essential trace element) between species of fish from Lakes Erie, Michigan and Superior. The range of copper in fish livers was from 1.5 to 28 mg/kg. The livers of lake trout from Lakes Superior and Michigan contained the highest levels. Lake whitefish (2.4 mg/kg) and bloater (2.4 mg/kg) from Lake Superior, smelt from Lake Michigan (1.5 mg/kg) and yellow perch (3 mg/kg) from Lake Erie contained the least copper. The smallest fish were analyzed whole, and again the copper varied little between alewives (Lake Michigan, 0.85 mg/kg), spottail shiner (Lake Michigan, 0.80 mg/kg; Lake Erie, 1.2 mg/kg) and trout perch (Lake Superior, 0.80 mg/kg). Trout perch in Lake Michigan had a relatively higher level, 2.7 mg/kg (Lucas *et al.* 1970).

Copper levels in dressed fish samples ranged from 0.50 mg/kg in lake whitefish from Moose Lake, Manitoba, an area free from industrial development, to 1.28 mg/kg (wet wt) in yellow perch from Lake Erie (Uthe and Bligh 1971).

Copper levels in the muscle of a number of fish species from the Fraser River, B.C., were generally low when compared to other areas and ranged from below the limit of detection to 1.53 mg/kg (wet wt), with an average of 0.54 mg/kg. The concentration of copper in the muscle decreased as the size of the fish increased (Northcote *et al.* 1975).

Toxicity of Copper to Fish

The toxicity of copper was related to soluble copper by Shaw and Brown (1974), who stated that the concentrations of Cu^{2+} and $CuCO_3$ together governed the toxicity, rather than the concentration of either of these forms alone. Chynoweth *et al.* (1976) discussed this and other aspects in their paper on research into copper toxicity. Most of the soluble copper in natural waters is present as ionic copper, copper carbonate and copper complexed with organic ligands. The percentage of these species of copper which are present in natural waters will vary with the quality of the water, thus they cannot be used individually in monitoring for copper or for setting an objective even if an analytical method for routine use was available. Also, the ultimate fate and the toxic effects of the different copper species cannot be fully determined with present scientific knowledge. Various studies looking at toxic effects and movement of copper through the organisms were discussed by Chynoweth *et al.*, but the actual mechanism of toxicity remained unknown. The predominant route of absorption of soluble copper is probably through the gills (see also under "Accumulation of copper in fish"). Further research into the effect of varying carbonate alkalinity, pH and hardness on copper toxicity was recommended, especially where alkalinity varies independently of pH and hardness. It was suggested that cations associated with hardness (Ca^{2+} and Mg^{2+}) reduced copper toxicity by reducing gill permeability to cationic transport and by competing with copper for active sites.

Pre-exposure to Copper

Dixon (1979) investigated the effect of pre-exposure to sublethal effects of copper on acute toxicity. Rainbow trout were exposed to 0.033, 0.095 and 0.14 mg Cu/L for 21 days. On days 0, 7, 14 and 21 of the sublethal exposure period, two 144-h acute tests were carried out using exposed and non-exposed fish. Pre-exposure to 0.095 to 0.14 mg Cu/L significantly increased the 144-h LC_{50}'s. Fish acclimated at 0.033 mg/L were more sensitive to copper than the controls. The degree of induced copper resistance was dependent on both the duration of pre-exposure and the concentration. This emphasizes that in the natural situation, the response of fish may differ depending on the nature of possible contamination by copper, whether it is from a continuous or intermittent discharge and whether the natural copper concentration is high enough to cause the above-mentioned resistance.

Chronic Toxicity and Sublethal Effects—Fish

Some sublethal toxicity effects are given in Table 4.

Reproduction—Fish

The survival, growth and reproduction of fathead minnows (*Pimephales promelas*) were similar in soft water (30 mg/L as $CaCO_3$) with copper concentrations of 0.0044

Table 4. Sublethal Toxicity of Copper to Fish

Species	Hardness mg/L as $CaCO_3$	Effect	Concentration mg Cu/L	Reference
Fathead minnow (*Pimephales promelas*)	200	Egg production significantly lower	0.037	Pickering *et al.* 1977
Fathead minnow (*Pimephales promelas*)	200	Spawning blocked	0.015–0.032	Mount 1968
Brook trout (*Salvelinus fontinalis*)	37.5	MATC	0.003–0.005	Sauter *et al.* 1976
Brook trout (*Salvelinus fontinalis*)	187	MATC	0.005–0.008	Sauter *et al.* 1976
Brook trout (*Salvelinus fontinalis*)	45	MATC	0.0095–0.0174	McKim and Benoit 1971
Catfish (*Catostomus* sp.)	37.5	MATC	0.012–0.018	Sauter *et al.* 1976
Catfish (*Catostomus* sp.)	187	MATC	0.013–0.019	Sauter *et al.* 1976
Bluegill (*Lepomis macrochirus*)	45	22 months MATC	0.021–0.04	Benoit 1975
Rainbow trout (*Salmo gairdneri*)	100	Teratogenesis	>0.001	Birge and Black 1979

to 0.0106 mg/L. Fry from parents exposed to these concentrations or unexposed parents grew equally well. The survival of fry was zero at 0.0184 mg Cu/L; half the adult fish were killed; and the growth and sexual development of the survivors were retarded. Exact measurements of the toxic form of copper were not made. In hard water it appeared that 0.033 to 0.0145 mg Cu/L had similar effects to 0.0184 to 0.0106 mg Cu/L in soft water (Mount and Stephan 1969). The authors estimated that a safe level of copper for fathead minnows was approximately twice as high as the safe level for invertebrates in water of similar hardness. Further investigations were recommended before the maximum acceptable toxicant concentration (MATC) and application factors could be determined precisely (Brungs *et al.* 1976).

Copper concentrations of 0.024 mg/L and below did not significantly affect the reproduction of fathead minnows (*Pimephales promelas*). Egg production was significantly lower at 0.037 mg Cu/L (hardness 200 mg/L as $CaCO_3$) and above. Reproduction was shown to be more sensitive than growth and egg hatchability. The length of the pre-spawning exposure time did not have a significant effect on reproduction (the number of eggs per female). The maximum acceptable toxicant concentration (MATC) was estimated to be 0.032 mg Cu/L, which would cause a 30% reduction in egg numbers. The unsafe level was estimated to be 0.034 mg/L. The copper concentrations were measured in experimental chambers using composites of seven daily water samples (Pickering *et al.* 1977). Waller *et al.* (1971) calculated that a 50% reduction in the number of eggs could be tolerated by a population of fathead minnows, but a reduction of 79% would cause population extinction. These figures were derived from a mathematical model that incorporated variations in environmental conditions and applied them to different levels of female fish mortality to determine the size of the spawning population.

There was a complete blockage of spawning for fathead minnows at copper concentrations between 0.015 and 0.032 mg/L (water hardness 200 mg/L as $CaCO_3$), but eggs transferred to and incubated in these concentrations hatched in numbers comparable with the controls. There was no relationship between copper concentrations and the ability of the males to care for the eggs; there were failures in every chamber including the controls. The growth rate was depressed by exposure to 0.095 mg/L, but after an initial depression at lower concentrations, fish grew more rapidly and compensated for the initial slow growth. Further work on second-generation fish is needed to determine the importance of decreased growth in the natural situation. The inhibition of spawning occurred at levels which had no effect on growth or caused no significant mortality, thus growth data cannot be used to estimate the MATC (Mount 1968).

A continuation of a 1971 study (McKim and Benoit 1971, see p. 29) indicated that concentrations of 0.0094 mg Cu/L or less had no adverse effects on either first- or second-generation brook trout exposed in the laboratory from eggs to spawning, though growth was retarded during the first 23 weeks of life. There was no accumulation of copper in the tissues and no toxic symptoms appeared during the 2 years when trout were exposed at 0.0045 to 0.0094 mg Cu/L. Copper was not passed from female to egg in fish exposed to 0.0094 mg Cu/L. Copper did accumulate slightly in or on fertilized eggs exposed to 0.0094 mg Cu/L over 3 weeks, but its location was not investigated. It might have been bound to the outer egg membrane and not incorporated into the egg. There was no direct toxic effect on the developing embryos and the initial accumulation was lost with time (McKim and Benoit 1974).

Sauter *et al.* (1976) studied the effects of low levels of copper on the hatching success, survival and growth of brook trout eggs and concluded that the MATC for copper for brook trout in soft water (37.5 mg/L as $CaCO_3$) lay between 0.003 and 0.005 mg/L. The MATC in hard water (187 mg/L as $CaCO_3$) was estimated to be between 0.005 and 0.008 mg Cu/L. Catfish were more tolerant and the MATC in soft water was estimated to be between 0.012 and 0.018 mg/L and in hard water between 0.013 and 0.019 mg Cu/L. Incomplete evidence was gained for walleye, but the MATC probably lay between 0.013 and 0.021 mg/L in soft water and was greater than 0.071 mg Cu/L in hard water. The difference in hardness did not appear to have a significant effect on the toxicity of copper. The MATC's were within the range determined in other studies.

Embryos and juveniles of eight species of fish were tested for survival against low levels of copper. Embryos were more resistant and the survival was only affected at the higher concentrations. Embryo mortality was almost complete at 0.5 mg/L for northern pike; 0.037 mg/L for rainbow trout; 0.333 mg/L for white sucker; 0.555 mg/L for herring, brook trout, lake trout and brown trout (late eyed); and at 0.111 mg/L for early eyed brown trout. Smallmouth bass embryos were not affected by exposure to copper (up to 0.5 mg/L). Significant reductions in the standing crop of juveniles of northern pike, rainbow trout and white sucker were found after exposure for 30 days from hatching to 0.104, 0.0317 and 0.0338 mg Cu/L, respectively. Standing crops of brook trout and lake trout were significantly reduced after exposure to 0.0435 mg Cu/L for 60 days. Copper concentrations

causing significant reductions in standing crop were dependent on the duration of embryo exposure; those exposed longest were the most sensitive. Herring and smallmouth bass control fish had high mortality, so that statistical analysis was impossible, but mortality data showed that copper concentrations of 0.1028 mg/L and above would have reduced the standing crop 30 days after hatching. Northern pike were more tolerant. Complete life cycle toxicity tests showed that the embryo-larval stage was adequate to establish the lowest effect concentration, and it was suggested that the best experimental period to estimate chronic toxicity would be the embryo-larval or early juvenile exposure for 60 days, for all species of fish. The results from one species could not be applied to another, as sensitivity varied quite widely (McKim *et al.* 1978).

Bluegills (*Lepomis macrochirus*) were exposed from juveniles through sexually mature adults for 22 months, then as eggs and larvae to 90 d post-hatch to a range of copper concentrations from 0.012 to 0.162 mg/L. The MATC lay between 0.021 and 0.04 mg Cu/L (hardness 45 mg/L as $CaCO_3$). Adult survival, growth and reproduction were unaffected at 0.077 mg Cu/L and below. At 0.04 and 0.077 mg Cu/L larval survival was significantly lower than that of the controls, but growth was not affected by 0.077 mg/L and below. Thus the most sensitive indicator of copper toxicity was larval survival. The application factor was calculated using the 96-h LC_{50} of 1.1 mg Cu/L and the MATC; it lay between 0.02 and 0.04 (Benoit 1975).

Rainbow trout embryos were as much affected by teratogenesis as by mortality when exposed to low concentrations of copper. Significant teratogenic impairment was caused by copper concentrations as low as 0.001 and 0.005 mg Cu/L. The skeletal system was the most sensitive indicator of teratogenic effects, and malformations included lordosis, scoliosis, kyphosis and rigid coiling of the vertebral column. Jaw defects were also common. Other fish species were less sensitive, but deformities occurred in channel catfish (at 0.5 mg Cu/L), goldfish and largemouth bass (5 mg Cu/L) (Birge and Black 1979).

Osmoregulation—Fish

Changes in blood osmolality in fish exposed to zinc or copper stress were observed by a number of authors. Freshwater fish displayed a lower serum Na^+, a decrease in blood osmolality and an increase in wet weight. Seawater-acclimated fish exhibited a higher serum Na^+, increase in blood osmolality and a decrease in weight compared with control fish (Courtois 1976). The exposure to copper ion caused a breakdown in normal osmotic homeostasis. The site of action for the toxicity of the copper ion was proposed to be in the gill at the Na^+-H^+ exchange site, where the copper ion caused a breakdown in water balance and loss in the control of the intake or loss of water through the gills. Further work was being conducted (Courtois 1976).

Physiological changes in fish affected their susceptibility to copper toxicity. The onset of smoltification, the physiological transformation which preadapts coho salmon to seawater existence, apparently increased their susceptibility to lethal effects of copper in fresh water, though the increase was small (96-h LC_{50}, 0.074 to 0.06 mg Cu/L). One of the physiological factors correlated with migratory behaviour in salmon is the elevation of activity in Na^+, K^+-stimulated ATPase in gill microsomes. This is thought to be important in the maintenance of the salt and water balance. The ATPase activity in fresh water and the percentage of survival in seawater decreased in proportion to the concentration of copper. The decreased ATPase activity probably contributed to the loss of osmoregulatory ability and death in seawater. Copper was shown to affect the growth, survival and condition of juvenile coho salmon at a level of 0.03 mg/L. Salmon survival in 0.02 mg Cu/L and less was similar to that of the control fish. Feeding ceased at the beginning of exposure to 0.02 and 0.03 mg/L; it was resumed after a while but only slowly at 0.03 mg Cu/L, while 0.01 mg Cu/L caused slight inhibition of feeding. The coefficient of condition (K) of the fish was the parameter that correlated most closely with copper concentration. The coefficient of condition ($K = 100\ W/L^3$ where W denotes weight in grams and L denotes fork length in centimetres) fell rapidly in 0.03 mg Cu/L for 10 weeks, levelled off and then increased slightly with the resumption of feeding. The percentage of downstream migrants was reduced when fish which had been exposed to copper even at the lowest (0.005 mg/L) concentration tested were released into a stream (Lorz and McPherson 1976, 1977).

Babine Lake, B.C., is part of the Skeena River system which has a large enhanced fishery resource. Two copper mines operate close to the lake and a study was conducted to determine whether there were any adverse effects of copper on sockeye salmon (*Oncorhynchus nerka*). The study described in the previous paragraph indicated that copper adversely affected juvenile coho salmon even at the lowest concentration tested under the specific water quality conditions of the test (0.005 mg Cu/L) (Lorz and McPherson 1976, 1977). Acute toxicity tests were done on site (Babine Lake) with sockeye fry, fingerlings and smolts. The 96-h LC_{50} values ranged from 0.21 to 0.22 mg Cu^{2+}/L for fry and 0.24 mg Cu^{2+}/L for smolts and fingerlings. The copper complexing capacity of Babine

Lake water was in excess of 0.1 mg Cu^{2+}/L, and this together with the low levels of copper present in the lake, 0.004 to 0.044 mg/L, indicated that the copper levels in Babine Lake posed no threat to the sockeye salmon fishery. Studies were also done to determine whether there were any sublethal copper effects which could affect fish overwintering in the lake or their tolerance to seawater after migration to the sea. Laboratory exposure of Babine Lake smolts for 144 h to 0.03 mg Cu/L in fresh water followed by 24-h and 48-h salt exposure showed that the smolts were unable to regulate plasma sodium levels. These results confirmed the results of other researchers showing that copper interferes with salinity tolerance in salmonids. The threshold range of copper which might disrupt the osmoregulatory capability of sockeye salmon was calculated to be in the range of 0.105 to 0.154 mg/L. The calculation took the complexing capacity of the lake water into consideration. This level of copper was well above the levels of copper which were present in the lake (Davis and Shand 1978).

Respiration—Fish

An initial increase and then a decrease in the oxygen consumption rate was demonstrated for juvenile bluegills exposed to 0.3 to 3 mg Cu/L and was positively related to the copper concentrations. After an initial increase, the respiratory rate decreased and returned to the base level after 14 to 18 h. The oxygen consumption had dropped below the normal rate by 24 h and remained depressed. The least depression occurred in fish exposed to 0.3, 0.5 and 1.0 mg Cu/L. Fish exposed to 0.1 mg Cu/L showed less than 1% increase or decrease from the control respiratory rate. Seven days after the copper had been flushed out of the system, all the fish had either recovered or showed strong evidence of recovery to the base oxygen consumption rate. The test bluegills exhibited a similar level of activity to the controls, so the increase in respiratory rate on exposure to copper was due to a greater respiratory demand and not an increase in swimming activity, as had been reported for other species (O'Hara 1971).

Feeding Behaviour—Fish

Lett *et al.* (1976) confirmed the findings of other investigators who stated that the initial response of fish to sublethal copper (and other heavy metals) was the immediate cessation of feeding, but that feeding was resumed and returned to the level of the control fish, the length of time depending on the dose and size of food ration. The rations were digested as efficiently by the copper-exposed fish (0.075, 0.150 and 0.225 mg Cu/kg ration) as by the controls.

The effects of low concentrations of copper (0.006 to 0.115 mg/L) on brook trout feeding behaviour, cough frequency and locomotor activity were studied by Drummond *et al.* (1973). The cough frequency response was greater and occurred within a few minutes of exposure to the higher copper concentrations of 0.055 and 0.115 mg/L. The frequency declined after the 20th hour of exposure. At 0.006 and 0.015 mg/L changes in frequency followed the same pattern, but the increases were smaller and developed slowly; no change was measurable until after the 4th hour of exposure. The difference in cough frequency was statistically significant between 0.009 and 0.006 mg/L and between 0.006 and 0.0 mg Cu/L. Locomotor activity increased within minutes of exposure to all concentrations of copper (0.006 to 0.115 mg/L). The activity lasted for 6 to 8 h at all concentrations and slowly subsided to the pretreatment level. Feeding was much less aggressive after exposure to copper and ceased after 2 h of exposure to 0.009 mg Cu/L and above. Nearly 40% of the fish in 0.006 mg Cu/L did not feed, but began to feed again in what appeared to be the normal manner within 24 h. Feeding at higher levels of copper did not return to normal. It was suggested that yearlings, which are available year round, could be used for the above tests and would be sensitive enough to indicate quickly whether there should be concern and thus further testing of the existing water quality. Although yearlings were not as sensitive as alevin-juveniles, statistical differences occurred between controls and the exposed fish within the range of the MATC of 0.0094 mg Cu/L.

Sellers *et al.* (1975) compared the effect of copper and zinc on cough frequency, ventilation frequency, and the amplitude of opercular and buccal respiratory pressure changes in rainbow trout after 6 h of exposure at near lethal concentrations. The changes were greatest at 0.04 to 0.06 mg Cu/L, and less at higher and lower concentrations. There was very little change in blood P_{O_2} and blood pH compared to the sharp decrease in both P_{O_2} and pH caused by zinc. Zinc causes death by hypoxia and gill destruction; copper has an effect on osmoregulation (see "Osmoregulation").

Hematology—Fish

Investigations on the effects of sublethal exposure to copper on various blood parameters were carried out by McKim *et al.* (1970) using brook trout (*Salvelinus fontinalis*). The changes were related to survival and reproductive success. There were short-term physiological changes in the red blood cell count, hematocrit, hemoglobin, plasma chloride, plasma glutamic oxaloacetic transaminase (PGOT), osmolarity and total protein on exposure to 0.038 to 0.089 mg Cu/L for 6 and 21 days.

The changes seemed to be transient and were roughly proportional to the concentration of toxicant. There were no detectable alterations in the blood during the long-term experiments of 337 days except for a statistically significant decrease in the activity of PGOT at concentrations of 0.0174 and 0.0325 mg Cu(II)/L (range, 0.0034 to 0.0325 mg/L). During the 21-d experiment, a 10% mortality occurred at 0.0692 mg/L and during the 337-d exposure, a 60% mortality occurred at the 0.0325 mg/L level. As the direction of change in PGOT activity varied, caution must be applied in the interpretation of the results.

Christensen *et al.* (1972) related the findings of a study on blood changes in the brown bullhead (*Ictalurus nebulosus*) in response to low levels of copper to tissue levels and effects on juveniles exposed for short (30 days) and long terms (juveniles to adult). There were large and significant changes in a number of blood parameters (hematocrit, hemoglobin, plasma glucose, plasma protein, chloride) after the 30-d exposure period. The parameters all returned to levels that were not significantly different after 600 d except for the activity of PGOT (plasma glutamic oxaloacetic transaminase). Insufficient spawning occurred, so that no fry or early juveniles could be exposed, therefore, a long-term "no effect" copper concentration could not be established. A similar experiment with brook trout was successful in this respect, and the results were used to develop a simple arithmetic relationship between the long-term and short-term biochemical responses of the trout. This relationship was used to calculate a "no effect" copper level for all stages of brown bullhead, assuming that the same relationship existed between the two species of fish. The value calculated for the bullhead from the brook trout factor was 0.011 mg Cu/L compared with 0.016 mg Cu/L calculated from the short-term and long-term blood data on the bullhead. Considering the many variables involved, the two values were quite close and are possible criteria for predicting safe environmental levels of copper for some fish species.

Comparing the effects of copper on blood parameters with those on survival, growth and reproduction, the levels of copper during chronic exposure which affected the survival, etc. did not cause measurable change in the blood, as a no-effect level for these parameters was concluded to be 0.023 mg/L in soft water (45 mg/L as $CaCO_3$). The MATC for brook trout was given as between 0.0095 and 0.0174 mg Cu/L (range of copper tested was 0.0034 to 0.0325 mg Cu/L), so measurements of the blood parameters using brook trout do not appear to give a good quick method for determining safe concentrations of copper (McKim and Benoit 1971), in contrast with the studies using bullhead described in the previous paragraph.

Endocrinology—Fish

A response to stress in fish, the increased secretion of corticosteroids, has been suggested as a possible parameter for a fairly rapid method of detecting the presence of pollutants (Donaldson and Dye 1975). The increased secretion, however, was only transitory at levels similar to those given as maximum acceptable toxicant concentrations (0.0094 mg Cu/L in soft water by Drummond *et al.* 1973) and the suggestion needs more study. Elevated levels of serum cortisol were caused by exposure to sublethal levels of copper but not cadmium. The increase in cortisol on exposure to copper was dose dependent. The cortisol level returned to more basal levels within 8 to 24 h, as the fish acclimated to the copper (e.g. 0.015 mg Cu/L). In other words, the cortisol level returned to prestress conditions even though this stress was still present. The cortisol level in fish exposed to 0.06 and 0.09 mg Cu/L increased significantly after 24 h and remained elevated until the end of the experiment. Fish resistance to a second stress, handling and confinement, was measured to obtain an index of reduced fitness. Fish from tanks containing no added copper and 0.09 mg Cu/L showed greatly increased cortisol levels due to stress, but survival was very different between the two groups. Fish that had been exposed to copper had median survival times of about 12 to 15 h, while the control group had no mortality after 30 h of confinement. Cortisol levels in copper-exposed fish were as high as titers produced by severe handling and confinement stresses. By contrast, exposure to cadmium caused death without an elevation of cortisol levels. Also, prior exposure to cadmium did not influence the response to the stress of handling (Schreck and Lorz 1978).

Lloyd (1961a), who studied rainbow trout (*S. gairdnerii* Richardson), suggested that the increase in the toxicity of copper in water with low dissolved oxygen concentrations, compared with that at higher oxygen levels, was caused by the increase in the rate of respiratory flow.

Growth—Fish

Growth reduction was a more sensitive indicator than LC_{50} for fish at the post-larval stage of development. The threshold concentration resulting in a reduction of growth for salmonids (*Salmo gairdneri*) was 0.015 mg Cu/L, in soft water, to 0.1 mg/L, in hard water, at pH 7.5 to 8. At a lower pH, the threshold in soft water was 0.01 mg/L and in hard water, 0.06 mg/L (Spear and Pierce 1979a).

The growth rates of rainbow trout (≈55 g) exposed in water of hardness 360 mg/L as $CaCO_3$ to copper con-

centrations ranging from 0.170 to 0.511 mg Cu/L were initially depressed. The rates had approached values for control fish within 40 d under conditions where ration size and activity of the fish were regulated (Lett *et al.* 1976).

Some Factors Affecting the Toxicity of Copper in Fish

The Effect of Fish Size on Toxicity

The effect of fish size on toxicity was investigated by Anderson and Weber (1975a) using mature guppies (*Poecilia reticulata*). The 96-h LC_{50} for mature males was 0.139 mg Cu/L for a 0.1-g fish and had decreased by a factor of 8 as the fish grew from newborn to sexually mature adults. Hedtke *et al.* (1978), working with coho salmon (*Oncorhynchus kisutch*), related body weight to toxicity and showed that the relationship could be very different at different stages of development. The mortalities were directly proportional to the concentration of the toxicant (Cu, Ni, Zn) and exponentially related to the body weight. The influence of body size was dependent on the species of fish, toxicant and the stage of development. The weight exponents were specific for each toxicant tested and changed both in magnitude and in sign (+ve or -ve) as the fish grew through the early developmental stages. This is probably due to the rapid and drastic physiological changes that occur in the early life of fish. A positive exponent, for example, meant that the heavier fish were less susceptible to the lethal effects of the toxicant. The range of lethal concentrations changed with the age of the fish, but during development the weight relationship seemed to function independently of the age factor. The 96-h LC_{50} for 56-d-old juvenile coho salmon was 0.06 mg Cu/L; for 130-d-old fish, 0.166 mg Cu/L; and for 151-d-old fish, 0.212 mg Cu/L. This again emphasizes the care that must be exercised when there is only a limited amount of toxicity information available, as data from one stage in the life history cannot be assumed to be relevant throughout the life of the fish. Not all experiments indicated a decrease in sensitivity with an increase in body weight. The 96-h LC_{50} for rainbow trout was approximately the same for large (176 g) and small fish (3.9 g wet wt), but at higher concentrations of copper, the larger fish were less tolerant and the survival time was shorter than for the juveniles. For example, 50% of the 176-g fish survived for approximately 19 h in 0.4 mg Cu/L and 50% of the 3.9-g fish survived for approximately 38 h in the same concentration of copper (Anderson and Spear 1980b).

pH

There are few studies demonstrating the effect of pH on the toxicity of copper, and the available literature is discussed in reports by the European Inland Fisheries Advisory Commission (EIFAC 1976) and Spear and Pierce (1979b). In general, copper is precipitated at alkaline pH and survival time is longer. Miller and Mackay (1980) showed that copper is the key factor causing mortality at pH 5.4, as no damage was reported in fish exposed to pH 5.2 in the absence of copper. An antagonistic interaction occurred, contrary to expectations, between copper and pH 4.7 and less. It was postulated that low pH stimulated greater mucus production by the gills when fish were exposed to equally toxic concentrations of both copper and pH than copper alone, and that the mucus chelated the copper even at a pH as low as 3.5 under the conditions of the experiment. Factors such as alkalinity and pH, as they effect copper toxicity, have still to be fully elucidated (see also "Hardness").

Temperature

EIFAC (1976) discussed papers which indicated that the relationship between temperature and copper toxicity was not straightforward. A crossing over of the concentration-response curves for copper toxicity to juvenile rainbow trout indicated differences in the effect of temperature depending on the concentration of copper and length of exposure. The survival time increased for fish exposed to acutely lethal concentrations of copper at a temperature of 6°C compared with survival at 15°C. When rainbow trout were exposed to levels of copper which killed 50% of the fish in 6 days, the survival time at the lower temperature was reduced by two thirds of the 6-d LC_{50} obtained at 15°C. Other studies indicated a reduced survival time for a number of species with increased temperature or no alteration in the LC_{50}'s at different temperatures.

Hardness

Copper has been shown to be more toxic at acute levels in soft water (Waller *et al.* 1971; Brungs *et al.* 1976; Sauter *et al.* 1976) and this was discussed by Spear and Pierce (1979b). The 96-h LC_{50} for fathead minnows (*Pimephales promelas*) was 0.46 mg Cu/L in water of hardness 200 mg/L as $CaCO_3$ (Pickering *et al.* 1977). The 96-h LC_{50} for rainbow trout (*Salmo gairdneri*) was 0.102 mg/L, for zebra fish (*Brachydanio rerio*), 0.149 mg/L, and for flagfish (*Jordanella floridae*), 1.27 mg Cu/L in water of hardness 350 to 375 mg/L as $CaCO_3$ (Fogels and Sprague 1977). The 96-h LC_{50} for juvenile steelhead trout (*Salmo gairdneri*) in soft water, 20 to 25 mg/L as $CaCO_3$, was 0.02 mg Cu/L (Chapman 1973). See Table 5.

Chakoumakos *et al.* (1979) studied the toxicity of a number of copper species using small (3 to 10 g)

Table 5. Acute Toxicity of Copper to Fish, 96-h LC_{50}

Species	Hardness mg/L as $CaCO_3$	96-h LC_{50} mg Cu/L	Reference
Coho salmon (*Oncorbynchus kisutch*)	88–99	0.060–0.074	Lorz and McPherson 1977
Coho salmon 56-d juvenile		0.06	Hedtke *et al.* 1978
130-d-old fish		0.166	Hedtke *et al.* 1978
151-d-old fish		0.212	Hedtke *et al.* 1978
Fathead minnow (*Pimephales promelas*)	200	0.46	Pickering *et al.* 1977
Rainbow trout (*Salmo gairdneri*)	350–375	0.102	Fogels and Sprague 1977
Steelhead trout (*Salmo gairdneri*)	20–25	0.020	Chapman 1973
Zebra fish (*Brachydanio rerio*)	350–375	0.149	Fogels and Sprague 1977
Flagfish (*Jordanella floridae*)	350–375	1.27	Fogels and Sprague 1977
Atlantic salmon (*Salmo salar*)	14	0.025	Carson and Carson 1972
Cutthroat trout (*Salmo clarki*)	205	0.367	Chakoumakos *et al.* 1979
Cutthroat trout (*Salmo clarki*)	26	0.0157	Chakoumakos *et al.* 1979

cutthroat trout (*Salmo clarki*). Nine different combinations of alkalinity, hardness and pH were used to determine LC_{50} values for Cu^{2+}, $CuOH^+$, $Cu(OH)_2{}^0$, $Cu(OH)_2{}^{2+}$, $CuHCO_3{}^+$, $CuCO_3{}^0$, and $Cu(CO_3)_2{}^{2-}$. The acute toxicity of copper correlated inversely with the hardness and alkalinity of the water. The effect of alkalinity on copper toxicity was thought to be partly due to its effects on copper speciation. Alkalinity and pH independently controlled the specification of copper. At a given alkalinity, hardness determined the LC_{50}, and at a given hardness, alkalinity determined the LC_{50}. When hardness and alkalinity were held constant, the pH of the water determined the toxicity of copper. The most abundant forms of soluble copper over all the conditions tested were dissolved $CuCO_3{}^0$ and $Cu(OH)_2{}^0$. The amount of Cu^{2+} increased as the alkalinity and pH decreased, and a similar change was observed for $CuOH^+$. Within the pH range tested, the concentration of $Cu_2(OH)_2{}^{2+}$ increased with a decrease in alkalinity and the concentration of $CuHCO_3{}^+$ increased with a decrease in pH. $Cu(CO_3)_2{}^{2-}$ was more abundant at high pH and high alkalinity. As the proportion of Cu^{2+} and the hydroxyl species increased, the LC_{50} decreased (i.e. the toxicity increased). The toxic forms of copper in the above series of experiments were Cu^{2+}, $CuOH^+$ and $Cu(OH)_2{}^0$. $Cu_2(OH)_2{}^{2+}$ may also be toxic, but it was present in very small amounts. Copper was least toxic at high alkalinity (178 mg/L as $CaCO_3$) and hardness (205 mg/L as $CaCO_3$) (96-h LC_{50}, 0.367 mg/L) and most toxic at low alkalinity (20.1 mg/L as $CaCO_3$) and hardness (26.4 mg/L as $CaCO_3$) (96-h LC_{50}, 0.0157 mg/L measured by cuprethol on 0.45-μm filtrate) using similar sized fish. $CuCO_3{}^0$ was not found to be toxic, in contrast with the work by Shaw and Brown (1974), who indicated that both Cu^{2+} and $CuCO_3$ were involved in the toxicity of copper rather than the concentration of either of these two forms alone. (See also under "Forms of copper in the environment.")

Further study is needed to understand fully the mechanisms involved in interactions between copper and calcium and magnesium ions.

Toxicity of Copper in the Presence of Other Pollutants

Lloyd (1961b) studied the toxicity of zinc and copper alone and of mixtures of the two, in hard and soft water. Both metals appeared to exert their toxic action in a similar way, as the curve of the logarithm of the median period of survival and the logarithm of the concentration of the mixture fitted both sets of experimental data. Over a period of 3 days in hard water (320 mg/L as $CaCO_3$) 3.5 mg Zn/L was equally as toxic as 1.1 mg Cu/L. In a mixture, a given quantity of one metal could be substituted for an equally toxic quantity of the other without altering the toxicity of the mixture. In soft water (15 to 21 mg/L as $CaCO_3$), 0.56 mg Zn/L was similar in toxicity to 0.044 mg Cu/L over 7 days. In a mixture, there seemed to be a more than additive effect at the higher concentrations which were tested, but with low concentrations of zinc and copper, the toxicity appeared to be additive. This points out that in rivers where copper and zinc are present together there is a chance of synergism occurring.

The toxicity of a copper and ammonia mixture was shown to be similar in action to that of copper and zinc and close to the value predicted from the sum of the fractions of the threshold concentrations of the individual toxicants, as the curve of log survival time-log concentrations of toxicants fell between the two curves for the individual toxicants (Herbert and VanDyke 1964).

Observations in the field on fish living in lakes affected by smelter fallout near Flin Flon, Manitoba, showed that populations in lakes containing up to 0.01 mg Cu/L and 0.09 mg Zn/L were not noticeably affected. Some changes were occurring over a long time span in lakes exposed to high levels of zinc in the ranges of 0.2 to 1.0 mg/L (Van Loon and Beamish 1977). Harmful effects were noted in the laboratory for a trimetal mixture at 0.027 mg Zn/L, 0.005 mg Cu/L and 0.004 mg Cd/L, and much of the reproductive impairment was attributed to zinc (Eaton 1973). The fish were able to tolerate higher concentrations of metals in the lakes in the study than they could under laboratory conditions.

The toxicity of zinc and copper mixtures was shown to be more than additive by Anderson and Weber (1975b), using guppies as the test fish. The toxicity of copper and nickel mixtures were found to be concentration additive. When copper was in the presence of three other toxicants —nickel, dieldrin and potassium pentachlorophenate (PCP)—the resulting toxicity was called "response addition," i.e. it was predictable by summing the magnitude of response evoked by the individual constituents of the mixture. Equations were given for predicting the effect of various combinations of toxicant concentrations.

Anderson and Weber (1976) further reported an enhanced toxic effect on guppies (*Poecilia reticulata*) in a mixture of zinc and copper. Although some studies have indicated an additive effect, in this case the lethal potency was increased by a factor of 2.5 above the effect predicted on the assumption of strict additivity. The authors also discussed the establishment of permissible levels for mixtures, the situation which is most likely to occur in rivers and lakes.

A copper and nickel mixture was shown to have a concentration additive effect (i.e. an additive effect determined by the summation of the concentrations of the individual metals in a mixture after adjusting for differences in their respective potencies) on the growth and food consumption of juvenile guppies (*Poecilia reticulata*) (Muska and Weber 1977).

A mixture of zinc, copper and nickel sulphates was demonstrated to be additive in its toxicity to rainbow trout (*Salmo gairdneri*) in soft water (Marking 1977).

The acute toxicity to rainbow trout of mixtures of copper and phenol; copper, zinc and phenol; and of copper, zinc and nickel were examined over 48 h using concentrations of each toxicant which theoretically contributed equally to the toxicity of the mixtures. It was found that the toxicities could be predicted by summation of the fractional toxicities of the individual components of the mixture (Brown and Dalton 1970).

Sprague *et al.* (1965) studied the effects of copper and zinc pollution from a mine on Atlantic salmon populations in the Miramichi River, N.B. There was a strong boundary between the lethal and non-lethal concentrations and this was called the incipient lethal level (ILL); concentrations slightly above the ILL were lethal in one day. The ILL for copper was 0.048 mg/L and for zinc, 0.6 mg/L in soft water (hardness 20 mg/L as $CaCO_3$). The copper and zinc did not potentiate each other as far as the ILL was concerned, as the toxicity caused by the two metals was additive in nature. Avoidance was studied in the laboratory using young salmon and in the field on fish passing through the counting gates on the Miramichi River; avoidance was correlated with copper and zinc levels in the river. The metal levels fluctuated owing to mining activity and river flow. Motivation to ascend the river would be present in the fish in the Miramichi River but not in the laboratory study fish. The salmon parr avoided a copper-zinc concentration totaling 0.07 ILL in favour of laboratory water with a concentration of 0.05 ILL caused by the naturally contained copper and zinc (0.002 mg Cu/L, 0.003 mg Zn/L). The minimum avoidance threshold, 0.07 ILL, may not prevent a fish from ascending the river to spawn. The salmon regularly ascended the river to spawn before the mine began operation, variations in movement being governed by river flow. The avoidance thresholds in the river were 0.35 (in the years 1961, 1963) and 0.43 ILL (1960). There did not seem to be any changes in the salmon's reactions to metal pollution over 4 years. Other effects of metal pollution were poor survival of parr and underyearlings and low numbers of eggs; 85 to 140 eggs/84 m^2 were present in affected reaches of river, much lower than the 200 eggs/84 m^2 required to maintain a salmon population. Salmon parr were found in the river below and opposite the entry of the tributary carrying mine waste, but chemical analysis showed that the contaminated water had not mixed with the river water and it was the latter that the fish were using. Macroinvertebrates were also affected by mine wastes. Blackflies and some midges became numerous, but mayflies were absent when the toxicity was 0.6 to 1.0 of the ILL for fish. Caddisflies were more tolerant than mayflies.

The presence of humic substances, suspended organic solids and domestic sewage decreased the toxicity of

copper quite considerably. Solids removed about 70% of the soluble copper within 1 to 2 h. In the absence of organic solids, at pH 7.45, between 70% and 80% of the added copper remained in solution for about 20 h. At pH 8, about 80% of the copper was removed from solution as inorganic complexed species within 2 h. An increase of humic substances from 0 to 0.9 mg/L increased the survival time of rainbow trout by 50% when exposed to 2.0 mg Cu/L. The median period of survival appeared to be positively linearly correlated with the concentration of humic complexed copper. The proportion of copper which was complexed with humic substances also increased linearly from 0 to 17.5% with increase in the concentration of humic substances from 0.9 to 4.5 mg/L. Copper complexes were shown to be of very low toxicity to fish compared with the toxicity of the copper ion. The percentage of such complexes in natural waters will vary widely and much of the total copper may not be soluble but be associated with particulate material. Information on toxicity, gained with natural water as the diluent water, cannot be applied universally to all waters (Brown *et al.* 1974).

Cook and Cote (1972) demonstrated that the presence of humic acids decreased the toxicity of copper considerably. Different concentrations of humic acid and copper were tested using Atlantic salmon (*Salmo salar*). The dilution water also contained zinc but copper toxicity predominated. The effect of humic acid was judged in terms of the number of standard toxic units required to achieve a lethal response. A toxic unit represented the concentration of the heavy metals that were just acutely toxic to salmon at a specific water hardness. Theoretically, one "standard" toxic unit would be lethal. The range of humic acid concentration—6, 10, 15, 20 and 30 mg/L—decreased the toxicity of copper; 2.45, 3.20, 3.20, 3.60 and 8.40 toxic units, respectively, were required to give a lethal response.

Carson and Carson (1972) also demonstrated the effect of humic acids on copper toxicity to Atlantic salmon. The 96-h LC_{50} for copper alone in soft water (hardness 14 mg/L as $CaCO_3$) was 0.025 mg Cu/L; with 5 mg humic acid/L, 0.09 mg Cu/L; and with 9.8 mg humic acid/L, 0.165 mg Cu/L. On a weight basis, lignosulphonate was not as efficient as humic acid in reducing the toxicity of copper. The 96-h LC_{50} for copper plus 17.6 mg lignosulphonate/L was 0.08 mg Cu/L.

Nitrilotriacetic acid (NTA) decreased the toxicity of a copper-zinc solution for small brook trout by a factor of up to 33 of the lethal threshold in a continuous flow situation. NTA was effective when its concentration was 3 or 4 times greater than the metal combinations (water hardness 14 mg/L as $CaCO_3$). The lethal threshold of copper for trout was about 0.05 mg Cu^{2+}/L and for zinc, 0.75 mg Zn^{2+}/L. The toxicity of mixtures was strictly additive under the conditions of the test (Sprague *et al.* 1968). The disodium salt of ethylenediamine tetraacetic acid (EDTA) also protected fish against copper toxicity. About six times as much EDTA as the metal was required (Sprague 1968).

The toxicity of copper to fathead minnows was tested using a natural water source as the diluent water. The stream, of hardness 274 mg/L (range 148-340 mg/L) as $CaCO_3$, received waste from a sewage treatment plant containing a variety of materials known to affect copper toxicity. The acute toxicity of copper, using total copper as the measurement, varied widely during the test period, 1.6 to 21 mg total Cu/L; the dissolved copper 96-h LC_{50} varied less, 0.60 to 0.98 mg Cu/L, indicating that the varying water quality was governing the amount of copper in solution. The authors also studied the effects of low concentrations of copper on reproduction and concluded that the MATC lay between 0.066 and 0.12 mg Cu/L, probably nearer to 0.066 mg/L (Brungs *et al.* 1976).

The addition of sublethal amounts of spent sulphite liquor (SSL) to diluent water taken from a soft water river reduced the toxicity of copper to Atlantic salmon parr. The 96-h LC_{50} of copper in the Exploits River, Nfld., hardness 8 to 10 mg/L as $CaCO_3$, was estimated to be about 0.125 mg Cu/L, much higher than for other waters of similar hardness. The difference was attributed to the presence of complexing agents in the water (humic acid, 4.5 to 5 mg/L). The addition of SSL further reduced the toxicity of copper: fish survived 0.18 mg Cu/L with 350 mg SSL/L, and there was only 10% mortality in 0.25 mg Cu/L with 450 mg SSL/L. The 96-h LC_{50} of SSL was 2000 mg/L. The pH was depressed by SSL and could have become a factor in the toxic effect at concentrations of SSL approaching the toxic limit. This chelating effect of SSL emphasizes the difficulty of predicting the toxicity of copper in natural waters from laboratory results, especially if water from the river or lake has not been used as the diluent water in experiments (Wilson 1972).

Avoidance of Copper

The attraction to and avoidance of copper by fish was investigated for a number of species. Shallow gradients of copper (0.011 to 0.017 mg Cu^{2+}/L) attracted goldfish and green sunfish, but channel catfish were not as strongly attracted. Largemouth bass did not react to the copper ions and the white sucker had a significant avoidance reaction (Kleerekoper 1973). When higher copper concen-

trations (0.025 and 10.0 mg/L) were used, goldfish entries into the copper zone were significantly less frequent (Westlake *et al.* 1974). Sprague (1964) demonstrated that Atlantic salmon (*Salmo salar*) parr avoided 0.004 to 0.005 mg Cu/L in the laboratory. Field observations at levels of about 0.05 of the lethal threshold concentration showed that adult salmon migrating upstream were turned back by higher concentrations, equivalent to 0.35 to 0.43 of the joint lethal threshold concentration of the mixture of copper and zinc present in the river. The incipient lethal level was 0.048 mg Cu/L and 0.6 mg Zn/L; when copper and zinc were both present, toxicity was additive (Saunders and Sprague 1967; see also Sprague *et al.* 1965 under "Toxicity of copper in the presence of other pollutants").

Low levels of copper (threshold level of 0.008 mg/L) depressed the olfactory response in fish (rainbow trout *Salmo gairdneri*) and could have an effect on behaviour such as homing into homestream water by adult anadromous fish. The concentration of ionic copper, the active form, was not determined, so it was possible that the threshold active concentration was lower than 0.008 mg/L. Also, the degree of depression increased with increase in exposure time; this could also lower the active concentration of copper, which would have an adverse effect (Hara *et al.* 1976).

Immune Response to Infection; Effect of Parasites

Roales and Perlmutter (1977) found indications that exposure to low levels of copper (0.009 mg/L) decreased the immune response of the blue gourami (*Trichogaster trichopterus*) to viral and bacterial infections. It was not known whether the effect was at the subcellular, cellular or organ level. A mixture of methylmercury and copper did not seem to lead to any greater or lesser immunological response to either antigen than when the two metals were alone. Rødsaether *et al.* (1977) suggested that copper in the water caused a lowered resistance of eels (*Anguilla anguilla*) to "red disease" caused by *Vibrio anguillarum*. The bacterium must have been present in the eels when they were caught in brackish water, since the organism is not viable in fresh water. Eels kept in fresh uncontaminated water (<0.006 mg Cu/L) remained healthy for 8 months. Forty eels were then transferred after 2 months in fresh water to water containing 0.03 to 0.06 mg Cu/L. The eels died from vibriosis within 50 to 60 days. A third group of 11 eels were transferred after 8 months in uncontaminated water to copper-contaminated water and they died from the same disease. *V. anguillarum* was isolated from the blood of the eels with symptoms of vibriosis, but no other bacteria were isolated from the blood. It was not conclusive that the disease caused the eels' deaths, but vibriosis symptoms were present in copper-exposed eels. The fish were stated to be relatively tolerant to heavy metals because of their rich slime production (Rødsaether *et al.* 1977).

Sockeye salmon smolts in Babine Lake, B.C., were heavily infested by the intestinal cestode parasite *Eubothrium salvelini* (35.3%). The infestation did not appear to be correlated with the sensitivity of sockeye to copper toxicity at acute levels, but it may have been responsible for the small size, poor nutritional state and unsuccessful seawater adaptation of some of the fish (Davis and Shand 1978). This is in contrast with the increased susceptibility to zinc of infested Babine Lake smolts (Boyce and Behrens Yamada 1977). The explanation for this difference is not known (Davis and Shand 1978).

Field Studies—Fish

A study of natural fish populations in two Norwegian lakes and the tributary streams, some affected by zinc and copper from old mine workings, was discussed in a UN report (EIFAC 1977). The data were not complete but some generalizations can be made. Fish were absent and the invertebrate community was low in the River Bjøraa, which drained the mine area. The 95th percentile values for zinc and copper concentrations in the river (0.405 and 0.230 mg/L, respectively) exceeded the proposed standards by factors of 3.1 and 5.5, respectively (soft water, 30 mg/L as $CaCO_3$). Spawning char avoided the inflow of the river Bjøraa to Lake Ringvatnet but spawned along the shores of the lake in shallow water (1 to 2 m). The quality and growth rate of the trout and char compared favourably with fish from other lakes in the same area of Norway that were unaffected by high copper loading. The two lakes supported good fisheries which were active year round even though copper exceeded the EIFAC standard for copper (0.005 mg Cu/L, 95th percentile; 0.001 mg/L, 50th percentile of soluble copper) by 1.9 and 1.2 times; the zinc concentrations were close to that of the standard (hardness 19 mg/L as $CaCO_3$). There were a number of factors which would have affected the copper toxicity. It was assumed that in the presence of humic substances in soft water, one-third of the copper might be in a form toxic to fish, but this project indicated that up to 86% of the copper was present as organic complexes and only 14% of the copper was toxic. A second factor was the acclimatization of the fish to copper, thus a viable fishery existed where it was thought that the concentrations of copper and zinc would be too high. The toxicity of copper and zinc together, assumed to be additive, may have been less than additive. A factor in the River Bjøraa, absent from the two lakes, was the presence of ferric hydroxide, which can be harmful to

fish at concentrations above 3 mg/L. There was also a low pH in the river, which slowly rose with distance from the mine from 4.7 at 0.5 km from the mine to 7.0 at the inflow to the lake (3.5 km from the mine). This study reinforces the necessity of determining the species of the metal which is toxic to fish and the effects of mixtures of metals under natural conditions. Fish populations may be affected by average concentrations, but they are more likely to be limited by sporadic high concentrations, which may persist for a sufficient length of time to cause harm.

Observations were made over a period of 4 years and 9 months in a stream experimentally stressed by the addition of 0.12 mg Cu/L. A number of species of fish moved out of the area and others were found congregating in parts of the stream where the concentration of copper was less than 0.1 mg Cu/L owing to the influx of spring or tributary water. One species, the orangethroat darter, seemed to be unaffected and spawned throughout the control and exposure areas during the three seasons that copper was added to the Shayler Run, Ohio, though sensitivity tests did not predict this. Bluntnosed minnows only spawned in the lower exposure area where the copper concentrations averaged 0.06 mg/L (range 0.035 to 0.077 mg/L); previous to the copper exposure the minnows had spawned throughout the area. A general effect of copper stress was a sevenfold reduction in the number of fry in the exposure area. Striped shiners and creek chubs were observed in prespawning activities in the exposure area but no spawning occurred. The effects on macroinvertebrates were discussed under "Field study—invertebrates." The only indirect effect of invertebrate changes on fish was a shift in diet, and no detrimental effects through the food web could be detected. The results of the acute bioassay tests varied in the laboratory (using stream water) and streamside because of variations in water quality, both chemical and physical, over time. The streamside results also varied because of changing stream conditions and showed a 40-fold variation for the fathead minnow and bluntnosed minnow. The 96-h LC_{50} (streamside) for the fathead minnow varied from 0.92 to 23.6 mg total Cu/L, and the 7-day LC_{50} varied from 0.56 to 23.6 mg/L. The toxicity was lower than expected, based only on hardness, alkalinity and pH of the Shayler Run water. The variation in LC_{50} values for dissolved copper was less than the variation in total copper values. The insoluble copper was relatively non-toxic, but all the soluble forms of copper could not be stated as being equally toxic. The sewage effluent discharged to the Shayler Run was thought to be detoxifying some of the copper introduced into the stream, as bioassays conducted using water from above and below the sewage plant effluent discharge indicated that copper was less toxic in a diluent water taken from below the sewage plant outfall. Detoxifying agents in the effluent and not changes in hardness or alkalinity reduced the toxicity. Orthophosphates were shown to form an insoluble complex and reduced the toxicity of copper in both standard test water and Shayler Run water. Streamside and chronic tests did not agree closely with stream observations for predicted unsafe concentrations. Gaps in the dilutions used in the streamside tests and biological and physical factors present in the stream probably caused these differences in values. The laboratory tests showed that 0.218 mg Cu/L prevented spawning, but in the stream the unsafe level was predicted to be 0.12 mg Cu/L for bluntnosed minnow spawning. The results of the Shayler Run and laboratory tests pointed out the difficulty of using application factors (AF) for predicting safe levels in a natural water. The Shayler Run water quality varied considerably, giving quite wide variations in copper toxicity (LC_{50} of 0.57 to 21 mg Cu/L). Applying the AF of 0.05 to 0.08 found in the standard water tests to the highest LC_{50} would give an estimated safe level of 1.1 to 1.7 mg/L, a level which was lethal during low flows. By applying the AF to the lowest LC_{50} a predicted safe level of 0.029 to 0.046 mg Cu/L is obtained. Variations in toxicity as wide as those found in the Shayler Run would probably not occur in waters not receiving an input of sewage and similar effluents, but this emphasizes the importance of other factors in influencing the toxicity of copper. The behaviour of fish is also important as they have been shown to avoid quite low levels of copper (Geckler *et al.* 1976). Avoidance of copper was studied by Sprague *et al.* (1965) (see under "Toxicity of copper in the presence of other pollutants").

Wildlife

Levels of copper in black and mallard duck primary feathers in the Sudbury area were highest in those caught nearest to the smelter and decreased with distance. Water, vegetation and precipitation were possible sources of copper (Ranta *et al.* 1978).

Rudd and Genelly (1956) commented that problems stemming from the use of copper sulphate as an algicide have arisen for fish but not for game animals.

Heavy metals as a possible cause of low hatchability of common tern eggs in populations near Hamilton, Ontario, were examined, but this could not be shown to be one of the main causes of embryonic mortality (Connors *et al.* 1975).

There was very little in the literature to conclusively show that copper was a problem for wildlife. The recommended level for aquatic life should protect wildlife from any detrimental effects owing to copper.

Recommended Limit for Aquatic Life and Wildlife

The toxicity of copper under natural conditions cannot be predicted accurately from extrapolated laboratory studies, as there are many variables acting on the metal and on the aquatic organisms.

Analytical methods need further refinement before the speciation of copper can be accurately determined in routine analyses. Because of this inability to analyze adequately the species of copper, the recommended limit is expressed as total copper. This will in itself give a safety factor, as only in soft water with very little complexing and precipitatory capacity will most of the copper be in the bioavailable form.

The use of phytoplankton data as criteria is limited because of different culture media and experimental conditions. Concentrations as low as 0.001 to 0.002 mg Cu^{2+}/L were shown to suppress photosynthesis in *Nitzschia palea*, and growth was affected by 0.005 mg Cu/L. Nitrogen fixation by blue-greens was reduced by the addition of trace amounts of copper (0.005 mg/L) to lake water containing low copper levels (mean, 0.0027 to 0.0037 mg/L), and photosynthesis was reduced to a lesser degree. There was a shift in community structure away from blue-greens to greens and diatoms by the addition of 0.005 mg Cu/L.

Newly hatched *Gammarus* did not grow to adult size in copper concentrations above 0.0046 mg/L and there was 60% survival at this level. Eighty per cent survived in 0.002 mg Cu/L. The level of no effect for *Hydra* lay between 0.0025 and 0.005 mg Cu^{2+}/L using a synthetic medium. As with the different culture media used in phytoplankton studies, the results cannot be directly applied to the natural situation.

The 24-d LC_1 for trout was 0.0018 mg/L and the 7-d LC_1 for toad embryos and larvae was 0.001 mg Cu/L. A two-year study using brook trout showed that 0.0045 mg Cu/L produced no significant effects. Teratogenesis was observed in rainbow trout exposed to 0.001 to 0.005 mg Cu/L. Salmon parr avoided 0.004 to 0.005 mg/L and an increase in coughing was caused by >0.006 mg/L.

The quality of the water and sediments, the forms of copper present and the presence of other toxic chemicals will affect the toxicity of copper. These factors must be known in order to use the copper guideline for setting specific objectives on individual bodies of water.

Copper has been shown to be less toxic under natural conditions, as organisms tolerated higher levels of copper in the natural environment than in the laboratory for a variety of reasons. The invertebrate community composition in the presence of 0.023 mg Cu/L was very similar to that of the control station, which had a mean of 0.009 mg/L. The presence of sewage and other organics ameliorated the toxicity of copper. Hardness was not a significant factor in sublethal toxicity. The maximum acceptable toxicant concentration in soft water lay between 0.003 and 0.005 mg/L and in hard water, between 0.005 and 0.008 mg/L, but acute toxicity was more severe in soft water. Hardness had little effect on copper toxicity to invertebrates.

Copper was shown to be toxic in mixtures of metals at concentrations below that which would be toxic if copper were acting alone in the water.

The nutritional status of organisms is important, as poor nutrition is an additional stress on animals exposed to copper.

Natural levels of copper in lakes and rivers in Canada vary. A mean of 0.002 mg Cu/L was found in the Kenora region of northern Ontario. The concentration in the Great Lakes varied between <0.005 and 0.007 mg/L, and in industrial areas, e.g. downwind of smelters in Sudbury, copper was 0.005 to 0.011 mg/L.

The recommended limit for copper is 0.002 mg total Cu/L, but the existing levels and the effects of local water quality must be taken into account when setting an objective for copper for specific bodies of water.

Water Supply for Livestock

Copper in the Animal Body

Copper is considered to be one of the essential elements for animals. The whole body copper content of most adult animals is around 2 mg/kg in the fat-free tissues. Newborn and very young animals normally have higher whole body concentrations than the adults of the same species. For example, the whole-body, fat-free tissue copper concentration in the adult pig is approximately 2.5 mg/kg, while the newborn has a concentration of 3.2 mg/kg. Similarly, the adult rabbit has a concentration of 1.5 mg/kg and the newborn rabbit has a concentration of 4.0 mg/kg (Underwood 1971). The concentration of copper in various organs depends on the species, diet, and age of the animal. Usually the highest concentrations are found in the liver and brain, followed by heart, spleen, kidney and blood. The copper concentration in brain tissue increases with age; it approximately doubles from birth to maturity (NAS 1977, p. 14; Underwood 1971).

The copper concentrations in the liver, spleen, lung and aorta decrease from birth to maturity.

Ruminants, especially, have a high capacity for hepatic storage of copper. Underwood (1971) quoted data showing that in sheep 72% to 79% of the copper was found in liver, 8% to 12% in muscle, 9% in skin and wool, and 2% in the skeleton. The normal copper concentration range in the liver of sheep, cattle, ducks, etc. is 100 to 400 mg/kg (dry wt), and in the liver of rabbits, cats, dogs, pigs, domestic fowl, turkeys, etc. the normal range is 10 to 50 mg/kg (Underwood 1971). Ewes receiving a diet of 4 mg Cu/kg of body weight (as copper sulphate) also accumulated the metal in the liver and this was passed on to their lambs. The copper concentration in the liver of these ewes was 1725 mg/kg (dry tissue), whereas the control animals had a copper concentration in the liver tissue of only 180 mg/kg. The copper concentration in the liver of a lamb of a copper-fed ewe was 510 mg/kg (dry tissue), while the average copper concentration in the livers of control lambs was 191 mg/kg with a range of 120 to 305 mg/kg (James *et al.* 1966). Reports of sheep livers containing up to 1600 and 3000 mg/kg have also been quoted (NAS 1977, p. 21). Using organs with such high concentrations for human consumption would be dangerous.

There are no exact numbers for the copper requirements of various animals. It is considered that 4 mg Cu/kg of diet is probably adequate for growing pigs and poultry, and concentrations of up to 250 mg/kg resulted in a slight increase in the rate of weight gain (Underwood 1971; Poupoulis and Jensen 1976). Small increases in the dietary content of zinc and iron were effective in preventing the detrimental effects caused by copper supplementation in some animals (Underwood 1971; Williams 1977; Okonkwo 1977).

In the United States, levels of up to 15 mg Cu/kg of diet have been considered safe for livestock, although this value did not recognize the importance of maintaining a proper balance between copper and molybdenum, especially in sheep (6 to 10 parts of copper per 1 part of molybdenum) (NAS 1977, p. 20).

For sheep the daily requirements range from about 1 to 10 mg/d (or 1 to 10 mg/kg of diet) depending on the sheep's intake of molybdenum and sulphate and the type of sheep (Underwood 1971; Williams 1977).

The suggested dietary copper requirements for various types of chickens and ducks ranged between 3 and 6 mg/kg (Muller *et al.* 1976).

Copper is a component of a number of enzymes, i.e. ceruloplasmin, tyrosinase, laccase, ascorbic acid oxidase, uricase, monoamine oxidase, dopamine-β-hydroxylase, and cytochrome oxidase, and as such, participates in a variety of enzymatic reactions (NAS 1977, p. 15).

Copper Deficiency

Naturally occurring, clinically significant copper deficiency is generally limited to sheep and cattle (NAS 1977, p. 60). Copper deficiency first manifests itself by a slow depletion of body copper stores. It eventually leads to a severe anemia which is of the same type as that caused by iron deficiency. Copper-deficient animals fail to properly absorb iron, which leads to iron deficiency (NAS 1977, p. 15). The copper enzymes ceruloplasmin and cytochrome oxidase are intimately involved in the metabolism of iron.

Besides the anemia mentioned above, copper deficiency leads to bone disorders, depressed growth, depigmentation of hair and wool, abnormal wool growth, neonatal ataxia, impaired reproductive performance (fetal death and resorption in rats; depressed estrus in cattle), heart failure, cardiovascular defects and gastrointestinal disturbances. Chronic (scouring) diarrhea in cattle has also been connected with copper deficiency (Underwood 1971). Copper deficiency was also thought to be responsible for poor growth rate in cattle (Lloyd Davies 1977).

Bone disorders, e.g. osteoporosis and spontaneous bone fractures, have been reported in rabbits, mice, chicks, dogs, pigs, foals, sheep and cattle. There is evidence of osteoporosis in the offspring of ewes fed on diets totally deficient in copper (NAS 1977, p. 16). A study of bones from six copper depleted Jersey calves reared from birth for 30 weeks on a low copper diet (1.5 mg Cu/kg of feed-dry wt) showed that copper deficiency contributed to the development of a general matrix osteoporosis and overgrowth of the epiphyseal cartilage (Suttie and Angus 1978).

The reduced activity of lysyl oxidase, a copper-containing enzyme, which is probably the primary cause of the cardiovascular disorders, results eventually in diminished tensile strength of the aorta and in the myocardium becoming friable (NAS 1977, p. 17).

In non-ruminant animals copper deficiency also leads to cerebral edema, cortical necrosis, and achromotrichia (absence of pigment in the hair) (NAS 1977, p. 18).

Acute Toxicity of Copper

Sheep are poisoned by ingesting copper chloride at concentrations of 20 to 100 mg/kg. The symptoms of acute copper poisoning are vomiting, excessive salivation, abdominal pain, and diarrhea with fluid, greenish-tinged feces. Death follows within 24 to 48 h (NAS 1977, p. 23).

Data quoted by Griminger (1977) showed that a single dose of 800 mg Cu (as copper sulphate) was lethal to male chickens within 48 h and that 160 mg/d was lethal within 10 to 34 days.

Chronic Toxicity of Copper

Ingestion of copper in excess of requirements leads to its accumulation in the tissues, especially in the liver. The copper levels which are tolerated without toxic symptoms vary with the animal species and the period of passive accumulation may range from a few weeks to one year (Kneale and Smith 1977). The symptoms of copper poisoning are weakness, trembling, loss of appetite accompanied eventually by hemoglobinemia, hemoglobinuria, and icterus. Morphological and histophysiological changes occur in the liver, kidney, spleen and even in the white matter of the central nervous system (NAS 1977, p. 23). The chronic toxic levels of copper for ruminants are in the range of 20 to 30 mg/kg, and those for non-ruminants (swine, rats) are over 250 mg/kg.

When the copper concentration in the liver of sheep or cattle went above a certain value, the metal was then released into the blood causing hemolysis and jaundice. Underwood (1971) quoted some copper intake levels that produced toxic effects, e.g. 80 mg Cu/kg of diet for young sheep, 2000 mg/d for 122 days in a steer. In Australia copper poisoning occurs under "natural" conditions when sheep graze on plants containing up to 50 to 60 mg Cu/kg grown on cupriferous soils. Young calves are almost as susceptible as sheep to copper poisoning (Kneale and Smith 1977). Another source that must be considered in the case of grazing animals is the soil. It has been shown that grazing cattle ingest 140 to 1400 g of soil per day, representing up to 10% of the dry matter intake of cattle and up to 30% of sheep (Thornton 1974, 1979).

Finnsheep rams fed on a diet supplemented with 45 mg Cu/kg (as copper sulphate) starting at 10 months of age died after 307 days on the diet. The control group receiving the unsupplemented basal diet containing 5.9 mg Cu/kg lived at least 377 days (they were slaughtered at that time). The mean liver concentration at slaughter or death was 58 and 1017 mg/kg (dry wt), respectively (Reynolds 1976). Nubian goats receiving oral daily doses (copper sulphate) of 8 to 32 mg Cu/kg of body weight developed diarrhea, jaundice, arching of the back, grinding of the teeth, subnormal temperatures and eventually death after 54 to 144 days (Adam *et al.* 1977).

Symptoms of copper toxicosis, e.g. hemolytic crises, also appeared in sheep at dietary levels of 8 to 10 mg Cu/kg when the molybdenum concentration was very low (0.5 mg/kg). When the diet was supplemented with a vitamin-mineral preparation containing no molybdenum, the toxic level of copper rose to 30 mg/kg or more (NAS 1977, p. 20). The toxic signs appeared only when the liver accumulated 3 to 15 times the normal level of copper or about 150 mg/kg (wet wt). The morbidity was around 5% and the mortality of affected animals was around 75% (NAS 1977, p. 23).

No significant differences were found in the copper plasma concentration, the growth rate and the live weight of swine fed on a diet supplemented with 125 mg Cu/kg as compared with a control group (Wilkinson *et al.* 1977). The average daily weight gain of growing-finishing pigs and the gain:feed ratio were significantly depressed by 250 mg/kg supplemental copper (Lillie *et al.* 1977). Poultry seems to be more resistant. Data quoted by the National Academy of Sciences (NAS 1977, p. 25) or that obtained by Jackson (1977a) or Poupoulis and Jensen (1976) showed a slight reduction in the weight gain of chicks at a dietary copper concentration of 350 mg/kg or higher, while turkeys tolerated a diet containing 676 mg Cu/kg (as copper sulphate). On a daily intake basis, mallards tolerated 29 mg Cu/kg of body weight and chickens tolerated 60 mg/kg of body weight (NAS 1977, p. 25). The minimum lethal dose of copper in chickens, pigeons, and ducks varied from 300 to 1500 mg/kg of body weight depending on the copper form and the type and condition of the bird.

A diet supplemented with 500 mg Cu/kg slowed the growth of male broiler chicks. Gizzard erosion was observed at a level of 250 mg Cu/kg of diet (Poupoulis and Jensen 1976).

The Interaction of Copper with Other Elements and Compounds in Livestock

The interaction of copper with molybdenum and sulphate ion is of primary importance in the pathogenesis of copper deficiency in ruminant animals. Molybdenum seems to impair the utilization of dietary copper even at concentrations below 5 mg/kg (Suttle 1973; Fisher *et al.* 1976). If copper levels are high, too much molybdenum (above 5 to 10 mg/kg) induces copper deficiency and too little molybdenum induces copper toxicity. Applications

of sulphur to the soil, 50 to 200 mg/kg, can alleviate the molybdenum-induced copper deficiency by decreasing the molybdenum concentration in forage tissue below the undesirable level of 5 to 10 mg/kg (Gupta 1979). When the ratio Cu:Mo falls below 2:1, clinical manifestations attributed to molybdenum poisoning (or to copper deficiency) can be expected in cattle unless the copper concentration in feed exceeds 13 to 16 mg/kg. The symptoms of this condition are emaciation, liquid diarrhea full of gas bubbles, swollen genitalia, anemia, and achromotrichia leading in many cases to the death of the animal. Chronic copper poisoning can occur in sheep with moderate intake of copper, e.g. pastures containing 10 to 15 mg Cu/kg, and very low levels of molybdenum (0.1-0.2 mg/kg) and sulphate (Gupta 1979). Apparently cattle are more susceptible than sheep to the combination of excess molybdenum and deficient copper in their diet (NAS 1977, p. 17ff.). When the copper level in the diet falls much below 8 mg/kg, then 1 to 2 mg/kg of molybdenum in the diet can become toxic. Non-ruminants, especially swine, are more tolerant to high levels, up to 1000 mg/kg of molybdenum (NAS 1977, p. 17ff.).

Data quoted by the National Academy of Sciences (NAS 1977, p. 18ff.) showed that supplemental ascorbic acid in the diet aggravated the copper deficiency in chicks, swine and rabbits.

Zinc and iron protected swine from the adverse effects of high dietary copper concentrations (250 to 750 mg/kg) and their deficiency tended to intensify the copper toxicosis (R. Hill 1977; NAS 1977, p. 23). Similarly, the liver copper concentrations were reduced by up to 40% in sheep receiving zinc-supplemented diets (220 and 420 mg Zn/kg of diet), with concomitant reductions in the extent of liver damage (Bremner *et al.* 1976). The presence in the diet of compounds containing cadmium and sulphur adversely affects copper absorption by ruminants (Williams 1977). High dietary cadmium (200 mg/kg) caused a decrease in the copper concentration in the muscles, bones and feathers of hens concentrating it in the kidneys and liver. Feeding a diet containing 75 mg Cd/kg to goats for up to 19 months (from 2 years of age until death) resulted in a decrease in the copper concentration in the liver of approximately 60%. The reduction in the copper concentration in the liver of their kids was even more severe, over 90%, resulting in a low life expectancy (Anke *et al.* 1970).

In a series of experiments with broiler chicks, the heaviest 3-week-old chicks were obtained with a diet containing 80 mg Fe/kg and 12 mg Cu/kg (McNaughton and Day 1977).

High dietary intake of calcium carbonate and ferrous sulphide reduced copper absorption in sheep, probably by increasing the intestinal pH and by forming the insoluble copper sulphide, respectively (Underwood 1971).

Copper has a protective effect on selenium toxicity. For example, single oral doses of 20 to 40 mg Cu/kg of body weight protected ponies from the fatal effect of high oral doses of selenium (6–8 mg/kg of body wt) (Stowe and Brady 1978). Similarly, dietary levels of 1000 mg Cu/kg counteracted the growth depression and prevented mortality owing to selenium in ducks. It was suggested that copper modifies selenium toxicity primarily by causing the accumulation of a non-deleterious compound in the tissues (Jensen 1975).

Silver is another copper antagonist. The addition of 10 to 25 mg Cu/kg of diet of chicks prevented the growth reduction, increased mortality, reduced hemoglobin levels and reduced aortic elastin owing to the addition of silver (10 to 200 mg Ag/kg of diet) to the diet (Hill 1976). The reverse is also true: the symptoms of copper deficiency were accentuated in the presence of silver (Hill *et al.* 1964).

Mercury did not reduce growth or increase the mortality of copper-deficient chicks, but had both these effects when the diet was supplemented with copper (Hill *et al.* 1964).

Recommended Limit for Copper in Livestock Drinking Water

The literature review showed that ruminants (e.g. sheep, cattle) are the most sensitive species to copper toxicosis. Symptoms of copper poisoning start to appear at concentrations of around 30 mg Cu/kg of diet (equivalent to approximately 30 mg/d) or at lower values when molybdenum content is very low. Thus for sheep the maximum safe level of copper has been estimated to vary from just above 5 mg/kg to perhaps 20 mg/kg. In cattle the maximum safe level has been estimated at 100 mg/kg (Neathery and Miller 1977a). In swine the maximum safe dietary level of copper has been estimated at 150 to 400 mg/kg. For growing chicks and turkeys the maximum safe dietary level of copper has been estimated at 250 and 500 mg/kg, respectively (Neathery and Miller 1977b).

The National Academy of Sciences (NAS 1974, p. 63) recommended a copper concentration limit in the drinking water for livestock and poultry of 0.5 mg/L. The Australian Water Quality Criteria recommended a limit of 0.5 to 2.0 mg/L (Hart 1974, p. 124).

Considering the above safe dietary intake concentrations for sheep and cattle, 20 and 100 mg Cu/kg, respectively, and the daily water requirements (4-15 L/d for sheep and 26-110 L/d for cattle – NAS 1974), it is recommended that the copper concentration in drinking water for livestock should not exceed 1 mg/L where there are sheep and cattle, and should not exceed 5 mg/L in all other cases.

Water Supply for Irrigation

Copper in Soils and Plants

As in humans and animals, copper is essential for plant life, being a component of some important enzymes found in plants, such as laccase, ascorbate oxidase, plastocyanin, tyrosinase and amine oxidases (NAS 1977, pp. 6ff). These enzymes are copper proteins and most of them are thought to participate in the electron transfer system of plants (oxidation-reduction type reactions in which O_2 is the electron acceptor). Copper is found in most plant tissues, but it is concentrated mainly in the green leaves and the germ of seeds (Sauchelli 1969).

The normal range of copper in plant tissues is 1 to 50 mg/kg (dry wt), which means that copper is among the least abundant of the known essential plant nutrients (NAS 1977, p. 10). Page (1974, p. 33) quoted a much narrower range and lower values, 4 to 15 mg/kg, for the normal copper concentration in plant tissues.

The range of copper concentrations in a variety of vegetables collected in British Columbia was 1 to 15 mg/kg dry plant), and the average copper concentration in soil ranged from 10 to 30 mg/kg (Warren *et al.* 1970). Oats, barley and in general grains and seeds, tomato and cabbage are among the plants with higher than average copper content, while wheat, corn and potatoes have lower than average copper concentrations (NAS 1977, p. 10ff.). Grasses, with an average around 5 mg/kg, tend to have lower concentrations than legumes, which average around 15 mg/kg. Hutchinson *et al.* (1974) found that copper (and other metals: Ni, Zn, Pb, Cd) concentrations in plant tissues were higher in the early summer (young crop) than in the mature crops. The copper concentrations ranged between 2.4 mg/kg in the cauliflower leaf to 26.1 mg/kg in lettuce root. Copper seems to be one of the elements which does not accumulate in plant tissues. Hodgson (1969) gave a value of 0.45 for the ratio of the copper concentration in plant tissue to that in soil (oven-dry basis).

A survey of the copper concentrations of herbage and its supporting soil on parts of the coast of New South Wales showed that levels of herbage copper ranged from 0.3 to 14 mg/kg and the EDTA extractable soil copper ranged from 0.2 to 32 mg/kg (Beavington and Wright 1977).

In a survey of soil and vegetation in the Sudbury (Ontario) mining and smelting region, Hutchinson and Whitby (1974, 1977) found that although the soil copper concentration increased by a factor of 50 to 100 in the vicinity of the smelter compared to a point 50 km away, the copper concentration in the foliage of three naturally occurring plant species increased only by a factor of 2 to 7, approximately. In a fourth species (*Deschampsia flexuosa* or wavy hair-grass) the increase was by a factor of 56.

The copper content of soils seems to vary over a wide range. For example, Delas (1963) listed results from several countries showing a total copper concentration range in the top layers of soils of 80 to 1280 mg/kg. The usual concentration in uncontaminated soils ranged from 20 to 50 mg/kg. In soil, copper can exist in primary copper minerals, mainly sulphides and sulphosalts, bound by the clay or iron oxides fraction (70-80% of the copper for some soils), bound by the humus fraction, and in soil solution chelated by soluble organic molecules or as copper ions. The relative amounts in each of these forms depend mainly on the pH and the soil characteristics. Copper is adsorbed or bound by soil colloids (clays and humus) more strongly than other cations, e.g. Ca, K, Mg or many divalent heavy metals (Baker 1974). The specific adsorption maxima, i.e. adsorption by soil colloids, that is, clay minerals, organic matter and free oxides, at pH 5.5 ranged between 340 and 5780 mg/kg of soil. The percentage of organic matter and of free manganese oxides were the dominant constituents contributing to the specific adsorption. The non-specific adsorption (cation exchange capacities) of the same test soils, ranging from 1310 to 16 300 mg/kg, exceeded the specific adsorption (McLaren and Crawford 1973). The data were insufficient to determine which adsorption process was the dominant one in controlling the copper concentration in soil solution. According to Delas (1963), copper accumulates on the soil surface, i.e. does not migrate in depth, and is more readily available on non-calcareous soils. Copper is also very strongly bound by organic matter and many copper deficiencies occur on soils with a very high organic matter content (Hutchinson 1979). It has been suggested (Petruzzelli and Guidi 1976) that the first step in complexing the copper could involve the strongest links with humic substances of high molecular weight. This copper would not be available to plants. When all linkages of this type are exhausted, copper is absorbed by organic material of lower molecular weight, which forms weaker bonds. This

copper would be available to plants. In addition some mechanisms of steric hindrance could contribute to the different states of availability of copper to plants. Soil animals, e.g. isopods and gastropods, might also play an important role in the conversion of total copper to available copper in soil. Although studies in woodland systems have shown that soil animals, with the exception of isopods, do not concentrate copper from the litter in their tissues, the passage of litter through their gut converts even tightly bound copper to soluble copper. It was calculated that the whole litter production could pass through the guts of mesofauna (Wieser 1979).

The copper content (digestion with conc. HNO_3) of 295 soils collected in Ontario ranged between 2.1 and 144 mg/kg with a mean of 25.4 (standard deviation, S = 21.5 mg/kg). Organic soils had the highest copper concentrations with a mean of 65.0 (S = 26.9 mg/kg), reflecting agricultural practices of adding copper sulphate to combat copper deficiencies and using copper fungicides on vegetables. The sandy soils had the lowest copper content with an average of 20.2 (S = 27.8 mg/kg). Copper concentrations on a farm situated close to a nickel-cobalt smelter ranged from 486 to 2190 mg/kg. Within the range given above, soils on which vegetables and field crops were grown tended to have lower copper content than fruit soils and organic soils, again reflecting the use of copper fungicides (Frank *et al.* 1976b). In another study Frank *et al.* (1976a) found that copper accumulated, but not to any large extent, in orchard soils treated with copper fungicides. Thus the mean copper concentration rose steadily from 21 to 63 mg/kg over a 70-year period. The fact that copper did not accumulate as much as other elements, such as zinc, cadmium, chromium or nickel, was also confirmed in studies carried out by Lund *et al.* (1976) on the movement of heavy metals below sewage disposal ponds. Different results were obtained in a study of the Holland Marsh region, an intensively cultivated horticultural area 60 km north of Toronto. The study revealed that the agricultural practices (heavy applications of fertilizer and pesticides) over the last 50 years had led to a marked increase in the surface concentration of copper from approximately 25 mg/kg to 130 mg/kg. The copper concentration decreased with depth: at 12 to 18 in. it was only 20 mg/kg (Hutchinson *et al.* 1974).

Hutchinson and Whitby (1973) analyzed soils from the Sudbury (Ontario) region and found that the total surface copper concentrations ranged from 31 mg/kg at about 53 km from a smelter to 2071 mg/kg at 1.9 km from the smelter. The copper concentration in water extracts ranged from "not detectable" to 59.5 mg/L. Soils collected at depths of 5 and 10 cm had lower concentrations than the surface soils, but they presented the same pattern with respect to decreased values with increased distance from the smelter and very low concentrations, 1% or less of the total concentration, in the water extracts.

The absorption of copper by plants takes place in the form of Cu^+ and Cu^{2+} (Murphy 1972). The availability of soil copper to plants depends on the soil type, its composition and texture, the microbial activity of the soil, pH, oxidation-reduction potential, moisture, rainfall and plant species. Copper is less available in soils with high humus or organic matter concentrations, or with heavy additions of lime which result in the complexing of copper. High concentrations of phosphate, manganese or zinc also lower the availability of copper to plants, apparently by competing with it for transport sites on the plant roots (NAS 1977, p. 11ff.; Lagerwerff *et al.* 1977). Baker (1974) suggested that "... ion uptake is related to ionic activities or the relative partial molar free energies of ions at the root surface."

According to Mitchell (1977), the water-soluble concentration of copper in soil (also cadmium and nickel) closely predicted the copper uptake by lettuce and wheat.

The acid-extractable (1 mol HCl/L) copper concentrations in various Alberta soils ranged between 1.8 and 7.4 mg/kg. The levels were higher in poorly drained soils and they were significantly correlated with the cation exchange capacity and with the organic matter content of the soils. The copper concentrations in grain (barley, wheat, oats) seeds ranged from 5 to 10 mg/kg and from 2.7 to 4.5 mg/kg in grain straw (dry wt). The copper levels found in seeds and straws were not related to the acid-extractable copper concentrations of the soils (Dudas and Pawluk 1977).

Data quoted by Fuller (1977, p. 69) on the copper concentration in podzolic soils of eastern Canada are as follows: 1.06 to 19.03 mg/kg exchangeable copper and 0.09 to 0.46 mg/kg water-soluble copper. The low availability of soil copper was also apparent from the results obtained by Bradford *et al.* (1975). In their study, soils with total copper concentrations of 14.3 and 44 mg/kg gave a saturation extract concentration of 0.06 and 0.09 mg/L, respectively.

The extractable (0.1 mol HCl/L) copper concentration increased from approximately 1.1 mg/kg to around 3 mg/kg after irrigation with wastewater effluent containing 0.068 mg Cu/L or with effluent with injected sludge containing 0.501 mg/kg, for a period of 10 years. During this period a total of around 38 kg Cu/ha or approximately 16 mg Cu/kg of soil were applied. Similarly, the copper concentration in soil increased from around 0.5 mg/kg

to 0.7 and 0.9 mg/kg after addition of a total of approximately 1.5 and 3 mg Cu/kg of soil, respectively, during a period of 10 years (Sidle *et al.* 1977). The copper concentration in a series of plants grown in a soil containing 68.9 mg Cu/kg ranged between 2.1 and 32.4 mg/kg. When the copper concentration in the soil was raised to 309.6 mg/kg by the addition of copper sulphate, the range of copper concentrations in plant tissues was 1.8 to 54.0 mg/kg (Hutchinson *et al.* 1974).

Although small when compared with the increase in the copper content of soil, a variety of crops grown in sludge-amended soil showed increased copper concentrations. The copper concentrations in tissues of plants grown in the control soil containing 17.5 mg Cu/kg ranged from 2 (in corn seed) to 26 mg/kg (in tomato fruit) with an average of 8.7 mg/kg. The corresponding concentrations in plants grown in the sludge-amended soil containing 325 mg Cu/kg ranged from 2 (corn seed) to 30 mg/kg (tomato fruit and cantaloupe) with an average of 12.1 mg/kg (McIntyre *et al.* 1977).

Application of liquid sewage sludge containing 1430 mg Cu/kg (dry basis) at a rate of approximately 35 mg Cu/kg of soil led to an increase in the copper concentration of rye from 3.9 to 11.7 mg/kg (dry wt). Similarly, the copper concentration in the tissue of sorghum-sudan grass increased from 5.1 to 14.1 mg/kg. The increase in the copper concentration of corn plants (stover and grain) grown after the rye and sorghum-sudan crop was negligible (Kelling *et al.* 1977). In general the total copper uptake by the rye, sorghum-sudan and corn crops amounted to only 0.1% to 0.2% of the copper applied as sludge to the soil. The DTPA (diethylenetriamine-pentaacetic acid) extractable copper concentration ranged between 0.8 and 14.6 mg/kg of soil or 16% to 47% of the metal added to the soil (Keeney and Walsh 1975).

Data quoted by Garrigan (1977) showed that the application rate of copper and other toxic elements in sludge can vary over a wide range without causing any toxic effects. Thus, for copper the range quoted by Garrigan was approximately 0.9 to 20 mg Cu/kg of soil.

The yield and the copper content of the fruit, root, and leaf for a series of vegetables (bean, okra, peppers, tomatoes, squash, turnips, radishes, kale, lettuce, spinach) changed very little or not at all when 26 to 37 mg Cu/kg were added to the soil as sewage sludge (Giordano and Mays 1977).

Copper accumulation, but not toxic effects, was observed in lettuce, pea pods and potatoes grown in sludge-amended soil. When the soil was treated with approximately 30 mg Cu/kg (as sewage sludge), the copper concentration in the potatoes increased from 8.6 to 19 mg/kg, in the pea pods from 4.5 to 11.1 mg/kg, and in the lettuce from 1.6 to 11.9 mg/kg. Very small or no increases were observed in radishes and carrots (Dowdy and Larson 1975). Copper supplementation of approximately 0.3 to 20 mg/kg of soil increased the yield of several plants such as spring wheat, eggplant, cotton, apples, corn, etc. The direct treatment of tobacco and vegetable seeds with 0.01% cupric sulphide solution also increased the yields (NAS 1977, p. 12ff.).

Copper Deficiency

Copper deficiency in plants is rare, but it can occur in soils with low copper availability. Cereal crops are the most sensitive to the concentration of copper in soil (Gupta 1979). In normal soils, copper deficiency is triggered at total concentrations below 6 to 8 mg/kg. In soils where copper is highly complexed, the minimum copper concentration to avoid copper deficiency increases to around 30 mg/kg (NAS 1977, p. 10ff.; Gupta 1979). Gupta (1979) quoted results showing that the severity of copper deficiency is related also to weather conditions and that the temperature affects the uptake of copper by plants. Dry, sunny years accentuated the copper deficiency and dull, moist seasons decreased it in field crops. Increased temperatures in experimental tanks where the pots were placed led to an increase in the copper content of corn and cotton shoots.

The symptoms of copper deficiency are chlorosis, withering of younger leaves, stunted growth, dwarfing of the flower heads, exanthema in fruit trees (death of apical bud, rosetting and multiple bud formation) (NAS 1977, p. 12). The colour of legumes and forage plants deficient in copper tends to be grayish-green, blue-green, or olive green (Sauchelli 1969). Good summaries of the symptoms of copper deficiency were written by Sauchelli (1969) and Reuther and Labanauskas (1966).

Copper deficiency can also affect the crop yield. For example, in South Australia a virgin Seddon gravelly sandy loam with a low copper concentration of 4 mg Cu/kg had a pasture yield of 3506 kg dry matter/ha. The application of 1 kg Cu/ha (or approximately 0.4 mg/kg) as copper sulphate over 7 years more than doubled the yield to 7183 kg dry matter/ha (Carter and Day 1977). Gupta (1979) quoted studies in which copper applications increased kernel yields of oats by 500%, barley by 180%, wheat by 38%, and sugar beets by 66%. An adequate supply of copper is critical during the early stages of development of cereals. If the plant does not receive enough copper before flowering, growth will be restricted

at maturity, no matter how much copper is subsequently applied.

Acute Toxicity to Plants

The 5-d acute copper toxicity threshold of lettuce grown in solution was approximately 1.0 mg/L. Increasing the metal concentration past this toxicity threshold resulted in a proportionate reduction of the root growth. The lettuce seedlings eventually died at a concentration of approximately 1.3 mg Cu/L (Berry 1977).

Chronic Toxicity to Plants

Page (1974, p. 33) suggested that tissue levels above 20 mg/kg (dry wt) are toxic to plants. Toxic effects were noted in oats and red clover at tissue concentrations of 18 to 20 mg/kg (Von Hodenberg and Finck 1975). Davis and Beckett (1978; see also Beckett and Davis 1977) reported that the critical copper concentrations in plant tissue ranged between 14 and 25 mg/kg (dry matter) for barley, lettuce, rape, rye grass and wheat. The normal concentrations in these plant tissues ranged between 9 and 11 mg/kg. Any increase in the metal concentration above the critical value reduced the crop yield approximately in proportion to the logarithm of the tissue concentration. The critical value itself was independent of the growing conditions, but the accumulation of metal in the plant tissue was dependent on them.

In terms of copper concentrations in the nutrient solutions, the critical levels ranged from 0.3 mg/L in rape to 17.7 mg/L in barley. Light chlorosis appeared in oat plants grown in a nutrient solution containing 2 mg Cu/L. At concentrations of 10 mg/L the plants were very chlorotic, and at 20 mg/L the plants were small and the leaves narrow. The roots were normal with 2 mg/L concentration but small with higher concentrations. At 12.5 mg Cu/L the plant height was markedly reduced (Hunter and Vergnano 1953). Copper at concentrations greater than 1 mg/L in nutrient solutions reduced the root growth of tomato and lettuce seedlings (Hutchinson and Whitby 1973).

Chlorosis of young expanding leaves and necrosis of severely chlorotic interveinal areas occurred when barley was grown in a nutrient solution containing 64 mg Cu/L (Agarwala *et al.* 1977). The additional copper also caused a significant reduction in the weight of the plants, increased the sugars and starch and decreased the protein nitrogen content of the barley plants.

Perennial rye grass grown in a culture solution containing 10 mg Cu/L showed signs of toxicity (blackened root tips). The roots of plants grown in solutions containing 1.0 and 10.0 mg Cu/L were smaller than those grown in more dilute or control solutions, but the shoots of these plants were about the same (dry wt). The copper content of both roots and shoots increased with increased copper concentration in the solution. After 21 days in the culture solution containing 0 and 10.0 mg Cu/L, the shoots had copper concentrations of 3.9 and 24.7 mg/kg (dry wt), respectively. The corresponding concentrations in the roots were 7.7 and 3263 mg Cu/kg. The proportion of copper found in the roots increased from 39.3% of the total copper in the plant for plants grown in the control solution to 98% for plants grown in the solution containing 10 mg Cu/L. Despite the high copper content of the roots the metal did not relocate from them to the shoots when the plants were removed from the solutions. The percentage of copper removed from the solution by the plants decreased rapidly with increasing concentration in solution. Thus for plants grown in solutions containing 0.01, 0.10, 1.00 and 10.0 mg/L added copper, the uptake represented 100%, 36%, 22% and 12% of the copper present. For plants grown in soil the proportions were much less, i.e. 10.0%, 0.5%, and 0.1% of the 9.5, 95.3 and 953 mg/kg added copper (Jarvis 1978).

Copper toxicity has been associated with total copper concentrations in soil ranging from 150 to 400 mg/kg (Baker 1974).

According to Delas (1963), the copper became toxic starting at levels of 25 to 50 mg/kg of soil for the most sensitive plants. In soil solutions the minimum toxic level was around 1 mg/L. The toxicity of copper was greater at pH values below 6 to 7 and in humid climates. A low soil exchange capacity also increased copper toxicity.

Baker (1974), reviewing the literature on the relationship between the copper in soil, water and plants, concluded that for adequate growth most crops needed a copper activity or effective concentration at the root surface of 0.02 to 0.04 mg/kg. Levels of 0.1 to 0.3 mg/kg were toxic to some plants, e.g. tobacco.

Copper applied at a rate of 80 mg/kg of soil (as sewage sludge) decreased the dry matter yield of red beets from 47.0 to 17.6 g per pot. In a field experiment the addition of 20 mg Cu/kg of soil (as sewage sludge) decreased the red beet yield from 69.5 to 58.2 t/ha and the celery yield from 65.0 to 56.7 t/ha. Additions of larger amounts of copper decreased the yield even further (Webber 1972). The soluble (0.5 mol acetic acid/L) copper content of soil was 14.5 mg/kg.

A significant depression in the yield of snapbeans in culture was observed when the HCl (0.1 mol/L) or DPTA-

extractable soil copper exceeded 20 mg/kg or when EDTA-extractable copper exceeded 15 mg/kg (Walsh *et al.* 1972). The corresponding copper tissue concentration was approximately 30 mg/kg.

Data quoted by Davis (1974) showed that the cucumber and snapbean crop yields were not affected where copper sulphate was applied to the soil in amounts of up to approximately 70 mg Cu/kg of soil. When the quantity was increased to approximately 220 mg/kg, the yield dropped by 75% to 85%. Mitchell (1977) found that the lettuce yield was reduced by approximately 0.1% for each milligram of copper per kilogram of soil and that overall tissue copper concentrations were little affected by the level of copper, or other metal, concentrations in the soil. On the other hand, zinc and cadmium concentrations in tissue were depressed, and nickel concentrations were increased, by increasing soil copper concentrations.

Synergistic effects on the bush bean concentration of phosphorus, iron and zinc, and plant weight were observed when copper and nickel, or copper, nickel and zinc were added to the soil or solution culture (Wallace and Romney 1977).

Another aspect of copper toxicity was reported by Mathur and Sanderson (1978). Investigating soil from the environs of Ste. Clothilde (Quebec), they concluded that adding copper to soil may inactivate some of the exocellular soil enzymes that normally contribute to the degradation and mineralization of soil organic matter. The acid phosphatase enzyme content and the rates of respiration (measured as carbon lost as CO_2 upon incubation at 21°C) were negatively correlated with the copper concentrations of the soil samples. The range of total copper concentrations in soil was 7 to 388 with an average of 99.6 mg/kg. The range of soluble copper concentrations was 3.2 to 144 with an average of 36.3 mg/kg. It has been suggested (Mathur and Rayment 1977) that copper application be investigated as a method of mitigating the mineralization and subsidence (the permanent lowering of the surface elevation of organic soils) of some organic soils. Similarly, biological and biochemical activity of forest soils was affected by the concentration of copper (and zinc) in the organic topsoil. Thus the decomposition of organic matter, the nitrogen and phosphorous mineralization rates and the activities of soil urease and acid phosphatase decreased linearly with the logarithm of the copper plus zinc concentrations in samples of the humus layer (Tyler 1975). The nitrogen mineralization rate, for example, was reduced to 0.5 of the background level at a copper concentration in the humus layer of about 300 mg/kg; some reduction was noted at approximately 60 mg/kg, which corresponded to 3 times the background level (Mathur and Rayment 1977).

Recommended Limit for Irrigation Water

The recommended maximum copper concentrations in irrigation water in the United States are 0.2 mg/L for continuous use and 5.0 mg/L for short-term use on fine-textured soils (Berry and Wallace 1974, p. 60).

Based on the toxicity levels in nutrient solutions and the accumulation of copper in soil, Pratt (1973) suggested a maximum copper concentration of 0.2 mg/L for continuous use on all soils. The same value was suggested in Australia by Hart (1974).

Assuming that 1 m^3 of water is used to irrigate 1 m^2 of land per year (Chaney 1973) and the copper concentration in irrigation water is 0.2 mg/L, then using a soil depth of 15 cm and a soil density of 1.7 kg/dm^3, it can be calculated that 0.8 mg Cu/kg of soil will be added each year. The literature review showed that copper starts to become toxic at soil concentrations of 25 to 50 mg/kg for the most sensitive plants and under soil conditions favourable to copper availability, such as low pH and low clay, organic, iron and manganese content. Therefore, if the background level of copper is negligible, it means that at an accumulation rate of 0.8 mg Cu/kg of soil per year, more than 30 years would be necessary to reach the minimum toxic levels. It is considered that this provides a good safety margin even when one considers that the background levels of copper are not likely to be negligible. Baker (1974) estimated that approximately 2.6 mg Cu/kg of soil can be applied for 5 to 10 years on sandy soils and for up to 60 years on soils with high cation exchange capacity without reaching toxic levels. It must also be noted that grazing animals commonly ingest soil, up to 10% of the dry matter intake of cattle and up to 30% for sheep, and the copper concentration in soil is thus of direct importance to livestock (Thornton 1979). The role of copper from soils in animal nutrition is not yet completely understood, but soil concentrations of up to 50 mg/kg should be safe.

It is recommended that for continuous irrigation on all soils or for intermittent irrigation of land used for cultivating copper-sensitive plants, such as vegetables, the maximum total copper concentration in the irrigation water should not exceed 0.2 mg/L. Where the water is used to irrigate areas that are less sensitive to copper, cereals for example, the copper concentration should not exceed 1 mg/L. For short-term use this limit can be increased to 5 mg/L.

Recreation and Aesthetics

Drinking water for cottages and camping areas will probably not be treated or will undergo a minimum of treatment. To ensure that the level of copper will not exceed that which has been recommended for public water supplies, the concentration should not exceed 0.5 mg/L as total copper.

NOTE: The permissible level of copper in water which is thought to be safe for aquatic life is lower (0.002 mg/L) than that recommended for raw drinking water supplies and would therefore safeguard humans using recreational waters for water contact sports.

Recommended Limit for Recreational Waters

The concentration of copper in water should not exceed 0.5 mg/L copper.

Industrial Water Supplies

Water quality requirements for industry vary widely, but most industries are capable of treating the raw water to reach a quality that is acceptable to the industry in question. When considering the level of copper, the industries of concern would be those involved in food and drink processing.

Recommended Limit for Industry

Water that is used during the processing and that is included in the final product must meet the same requirement as for potable water supplies, therefore, a maximum concentration of 0.5 mg Cu/L is recommended.

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